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MALARIA IN TRAVELLERS AND MIGRANTS: DISEASE SEVERITY AND LONG TERM EFFECTS

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Malaria in travellers and migrants: Disease severity and long term effects

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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as well as online -for link please see www.ki.se

“If you want to walk fast walk alone, if you want to walk far, walk together!”

– African proverb, cited at the Pan African Malaria Conference, Dakar, April 2018

ABSTRACT

Malaria is a potentially fatal infection, where prompt and correct management is crucial for the outcome. Despite international efforts to reduce transmission, there are over 200 million new malaria cases with approximately 400 000 deaths per year, the majority occurring in Sub-Saharan Africa. Globalization, in terms of travel and migration, brings malaria also to non-endemic countries. Approximately 100-300 cases of malaria are diagnosed in Sweden per year, with significant variations during the last years due to changes in travel and migration patterns. Despite a well-established national surveillance, the clinical presentation, outcome and risk factors for severe malaria have not previously been described. Individuals growing up in endemic areas, gradually develop immunity, first to the most severe forms of malaria and eventually also to mild disease. However, how long protection against severe disease is maintained in individuals that are no longer exposed is not clear. Moreover, non-communicable diseases and obesity increase globally, including in travellers and populations living in malaria endemic areas, but the impact of comorbidities on the severity of malaria has not been systematically assessed. In addition to the well-described acute complications, malaria may cause long-term consequences that have been much less investigated. The association between malaria and Burkitt lymphoma in African children is since long recognized, but it is unknown if malaria is related to other cancer forms.

In this thesis, we have investigated risk factors for severe disease in travellers and migrants diagnosed with malaria in Sweden 1995-2015. Moreover, by using the Swedish surveillance system and national health and demographic registers, we studied long-term consequences of malaria in terms of lymphoma and other cancer.

In **Study I,** we assessed the risk of severe *P. falciparum* in immigrants from Sub-Saharan Africa and non-immune travellers. We showed that duration of residency in a malaria-free country was associated with the most severe manifestations of malaria. Immigrants who had lived for ≥15 years in Sweden had a similar risk of severe disease as travellers with origin in non-endemic countries, suggesting that immunity to severe malaria is lost after many years without re-exposure.

In **Study II,** we observed higher prevalence of chronic diseases among patients with severe malaria, and demonstrated that diabetes, obesity and combinations of metabolic risk factors strongly associated with severe malaria, both in non-immune travellers and migrants from endemic areas.

In **Study III,** we identified age ≤5 and ≥40 years, pregnancy, HIV, non-endemic origin and health care delay as risk factors for severe disease in *P. falciparum* malaria. In non-*falciparum* episodes, age >60 years, health care delay and endemic origin were identified as risk factors for severe disease. Newly arrived migrants were found to be a risk group for both severe *P. falciparum* and non-*falciparum* malaria. Moreover, the current WHO criteria for severe malaria did not reflect severity of disease in this non-endemic setting.

In **Study IV,** we demonstrated that malaria was associated with an increased risk of lymphoid neoplasm, especially B-cell lymphoma, in individuals born in malaria endemic areas diagnosed with malaria in Sweden. There was no increased risk of cancer overall, nor did single malaria episodes in travellers confer an increased risk. The results suggest that repeated exposure to malaria during childhood may lead to development of lymphoid neoplasms later in life.

In conclusion, this thesis has identified new risk factors for severe malaria and potential long-term consequences in travellers and migrants, with implications for improved strategies in the management, follow up and prevention of malaria, both in imported cases and in populations at repeated risk of infection.

LIST OF SCIENTIFIC PAPERS

I. Färnert A, **Wyss K**, Dashti S, Naucler P. **Duration of residency in a non-endemic area and risk of severe malaria in African immigrants**. Clinical Microbiology and Infection. 2015;21(5):494-501.

II. **Wyss K,** Wångdahl A, Vesterlund M, Hammar U, Dashti S, Naucler P, Färnert A. **Obesity and diabetes as risk factors for severe *Plasmodium falciparum* malaria: Results from a Swedish nationwide study**. Clinical Infectious Diseases. 2017;65(6):949-58.

III. Wångdahl A, **Wyss K,** Saduddin D, Bottai M, Ydring E, Vikerfors T, Färnert A. **Severity of *Plasmodium falciparum* and non-*falciparum* malaria in travelers and migrants: A nationwide observational study over 2 decades in Sweden**. Journal of Infectious Diseases.2019;220(8): 1335-45.

IV. **Wyss K,** Granath F, Wångdahl A, Djärv T, Fored M, Naucler P, Färnert A. **Malaria and risk of lymphoid neoplasms and other cancer: A nationwide population based cohort study.** BMC Medicine, accepted for publication 21st of August 2020.

Other relevant publications and manuscripts:

Sondén K, **Wyss K,** Jovel I, Vieira da Silva A, Pohanka A, Asghar M, Homann MV, Gustafsson LL, Hellgren U, Färnert A.
High rate of treatment failures in nonimmune travelers treated with artemether-lumefantrine for uncomplicated *Plasmodium falciparum* malaria in Sweden: Retrospective comparative analysis of effectiveness and case series. Clin Infect Dis. 2017 Jan 15;64(2):199-206.

Ljungberg J, Wångdahl A, **Wyss K**, Färnert A.Ljungberg J, et al. **Management of Malaria in Sweden**. Lakartidningen. 2019 Aug 12;116:FL9H

Jun-Hong Ch'ng, Moll K, **Wyss K,** Hammar U, Rydén M, Kämpe O, Färnert A, and Wahlgren M. **Enhanced virulence of *Plasmodium falciparum* in diabetic patients.** Manuscript under submission.

CONTENTS

LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme
ACT	Artemisinin-based combination therapy
adj	adjusted
AID	Activation-induced cytidine deaminase
AKI	Acute Kidney Injury
ALL	Acute lymphoblastic leukemia
BMI	Body mass index
CD	Cluster of differentiation
CFR	Case fatality ratio
CI	Confidence interval
CLL	Chronic lymphatic leukemia
DNA	Deoxyribonucleic acid
eBL	Endemic Burkitt Lymphoma
EBV	Epstein-Barr virus
ECDC	European centre for disease prevention and control
GFR	Glomerular filtration rate
HIV	Human immunodeficiency virus
HHV-8	Human herpesvirus-8
HR	Hazard ratio
HRP2	*Plasmodium. falciparum* histidine-rich protein 2
ICU	Intensive care unit
ICD	International Statistical Classification of Diseases and Related Health Problems,
InterLymph	International Lymphoma Epidemiology Consortium
iv	intravenous
LDH	Lactate dehydrogenase
OR	Odds ratio
NCD	Non-communicable disease
NPR	National Patient Register
P.	*Plasmodium*

PCR	Polymerase chain reaction
PfEMP1	*Plasmodium falciparum* erythrocyte membrane protein 1
SSA	Sub Saharan Africa
TPR	Total population register
VFR	Visiting friends and relatives
WHO	World Health Organization

1 INTRODUCTION

1.1 EPIDEMIOLOGY

1.1.1 Global burden of malaria

Malaria constitutes a major global health problem with 3.2 billion people, nearly half the world's population, living at risk of infection. According to the latest World Malaria Report, an estimated 228 million new infections and 405 000 deaths occurred due to malaria in 2018 [1]. During the years 2000-2014 there was a significant decline in the number of malaria infections and deaths, which unfortunately ceased during the last 5 years. Nonetheless, an increasing number of countries have been able to eliminate malaria; between year 2000 and 2018, nineteen countries with previously ongoing malaria transmission reported zero locally acquired cases [1]. Part of these progresses have been attributable to malaria-control interventions such as distribution of insecticide treated mosquito nets, indoor residual spraying, intermittent preventive treatment, improved diagnostics and access to treatment with artemisinin-based combination therapies [2]. However, there is evidence that the decline in malaria incidence and mortality started already before the introduction of malaria control measures [3-5]; some of the progress is likely explained by urbanization and economic development leading to better housing, healthcare access, and overall improvement of health status in the population.

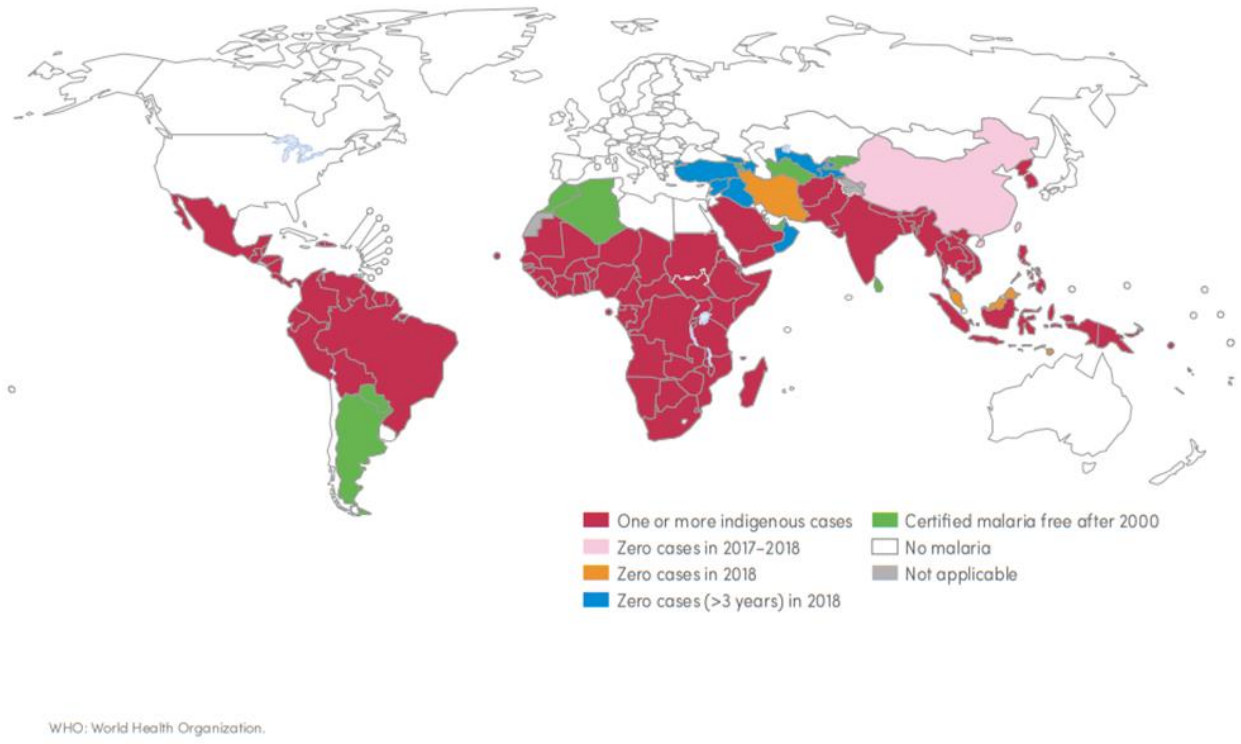

Figure 1: Countries with indigenous malaria cases in 2000 and their status by 2018.
Source: World malaria report 2019. Geneva: World Health Organization; 2019. Licence: CC BY-NC-SA 3.0 IGO.

In spite of the advances that have been made, major challenges remain. Of importance, 90% of all cases and deaths occur in the African Region, with 15 countries attributing to 78% of deaths, and in these countries, the decline in malaria incidence has not been as pronounced as in the rest of the world [6]. The hunt for an efficacious vaccine has finally produced results, and we now have a vaccine targeting the pre-erythrocytic stage of the parasite that is being implemented in four African countries, with over 275 000 doses administered to children by April 2020 [7]. However, long-term follow up from previous trials have shown a fast decline in efficacy, from 30-50%, depending on age-group, at one year after 3-dose vaccination [8], to only 4-7% after seven years [9], with possibly improved efficacy with a fourth booster dose [8].

Estimating incidence and mortality of malaria is evidently associated with substantial uncertainty and reports suggest both under- and overestimations of the numbers presented by WHO [10]. Irrespectively, the estimated malaria incidence and mortality might just be the top of the ice-berg since there are potential long-term effects of malaria including associations with Burkitt´s Lymphoma [11] and neurocognitive sequelae [12, 13]. Long-term sequelae have been insufficiently studied and are usually not included in estimations of malaria related morbidity and mortality. In addition, malaria may be associated to additional deaths and complications that are not directly attributable to the infection itself, such as; increased risk of bacterial infections [14], pregnancy and perinatal complications [15], reduced vaccine responses [16], exacerbation of TB [17] and increased viral loads in HIV infected [18].

1.1.2 Global burden of non-communicable diseases

Non-communicable diseases (NCDs) are increasing worldwide and have overridden communicable diseases as the leading global cause of death. In 2015, NCDs were responsible for 39.8 million (71%) of global deaths with cardiovascular diseases accounting for the largest group [19]. The increase in NCDs affects all countries, and 3/4 of all deaths due to NCDs occur in low and middle income countries [20]. One of the main risk factors for NCDs; obesity, is also increasing rapidly, with a doubling of prevalence between 1980-2014 [20], and has been entitled a “global epidemic” by WHO [21]. In Sub-Saharan Africa, the prevalence of obesity is increasing especially among women and children [22]. At the same time, pregnant women and children are the two groups most affected by malaria. Resource poor settings are especially vulnerable to the consequences of NCDs and are facing a double burden of disease as they continue to deal with potentially fatal infectious diseases like malaria, tuberculosis and HIV. Probably, we have not yet seen the full extent of this combination, and research investigating possible effects of the overlap in non-communicable and communicable diseases is much warranted.

1.1.3 Malaria in travellers and migrants

Due to migration and global travelling, malaria is also an issue in non-endemic areas, with increasing numbers in several countries, despite effective chemoprophylaxis and global achievements in malaria reduction [23, 24]. Throughout this thesis, the term “imported

malaria" is used for cases that are acquired in a malaria-endemic country and that are diagnosed and managed in a country without ongoing malaria transmission. Malaria is regarded as the most common imported potentially fatal infection in travellers returning from the tropics [25]. In the last years, the majority of imported cases constitutes of individuals originally from endemic countries, that have been visiting friends and relatives in their home country, a group often denoted "VFR" [26-28]. In addition to epidemiological publications, international surveillance systems such as the European Centre for Disease Prevention and Control (ECDC) have assisted the monitoring of numbers and trends of imported malaria. An estimated 30 000 travellers from northern countries, mainly in Europe and the North Americas contract malaria each year [29], and approximately 8000 cases of malaria have been reported annually in Europe during the last 5 years, with over half of the cases diagnosed in France and the United Kingdom [24]. In addition, a few autochthonous cases have been reported during the last years from Spain, Netherlands, France, Italy and Greece [30-33]. Except for Greece that has had reoccurring smaller outbreaks of locally transmitted *Plasmodium vivax* [34], occasional autochthonous cases in Europe are suspected of so called suitcase or airport malaria, or through other modes of transmission such as blood transfusion, organ donation, and sometimes non-determinable hospital-acquired infections. Hence, clinicians need to be aware of rare but possible malaria also in patients, without previous travel to malaria-endemic areas [35] [36].

1.1.4 Malaria in Sweden

In Sweden, malaria is a notifiable disease under the Communicable Diseases Act and all cases should be reported to the National Surveillance system at the Public Health Agency of Sweden, both by the treating clinician and the diagnosing microbiology laboratory.

The number of notified cases increased during the 1980-ies from approximately 100 cases per year to around 200 cases per year in 1995-1998, as a consequence of increased migration and traveling as well as inefficient prophylaxis [37]. Since then there has been a steady decline in the annual reported number of malaria cases and since 2005 it has again been around 100/year. The decrease has mainly been observed among travellers of Swedish origin, following changed recommendations for malaria chemoprophylaxis in 1999 [37]. In 2014, there was a dramatic increase in imported malaria in Sweden with 354 reported cases, and 249 reported for 2015, explained by an increase in Eritrean refugees during these years in combination with an exceptional high incidence of *Plasmodium vivax* infections in this group [38]. Several other European countries also reported an increase in *P. vivax* cases during these years, however the notification rate for Sweden was the highest among all European countries in 2014 [36]. After 2015 approximately 150-200 cases have been reported annually in Sweden, with a slight increase during the last two years [37].

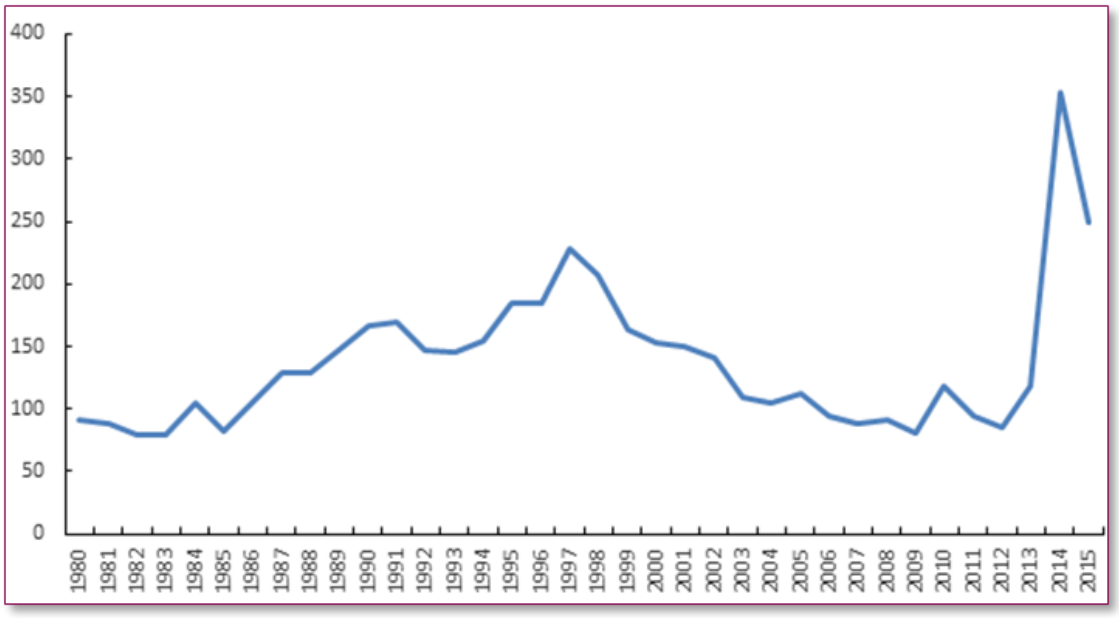

Figure 2: Reported cases to the Public Health Agency of Sweden per year 1980-2015.
Source: Folkhälsomyndigheten, Sjukdomsstatistik, Malaria.

The sensitivity of the Swedish surveillance system for communicable diseases has according to earlier studies been evaluated as very high, especially for diagnoses with parallel clinical and microbiological reporting [39]. A limitation with the surveillance data at the Public Health Agency of Sweden is, however, the lack of information on clinical presentation, treatment, detailed travel history and outcome. There is also uncertainty regarding sociodemographic characteristics such as patient origin.

1.1.5 Transmission intensity and endemicity

As will be discussed more in detail below, the clinical presentation of malaria is very much related to previous exposure, which in turn depends on transmission intensity and endemicity in the area where an individual is living. Previously, the intensity of transmission was estimated by assessing the proportion of children with enlarged spleens in an area. Today the parasite rate is used, meaning the prevalence of peripheral blood-stage infection in children aged 2-9 years [40]. Traditionally four terms have been used to categorize endemicity: Holoendemic (>75% parasite/spleen rate in 2-9-year-old children), hyperendemic (50-75%), mesoendemic (11-50%), hypoendemic (≤10%) corresponding to the today more often used terms high, moderate or low transmission [41]. These are evidently very crude measures of endemicity and within a country there is considerable variation and clustering, there can even be measurable differences between households [42].

Malaria transmission is characterised by how stable it is over time. In large parts of Sub-Saharan Africa, malaria transmission is stable over several years., and people are continuously exposed to malaria infection, however often with some seasonal variation. In contrast; epidemic transmission, more common in Asia and Latin America, is characterized by unstable transmission with significant fluctuation depending on season and region. The

entomological inoculation rate (EIR), the number of infected bites per person per year is a more direct method used to estimate level of transmission and also captures fluctuation in transmission [43].

1.2 THE PARASITE

1.2.1 *Plasmodium* species and transmission

Malaria is caused by the protozoan parasite belonging to the genus *Plasmodium* and is transmitted from one person to another by the *Anopheles* mosquito. Transmission has also been observed to occur occasionally through blood transfusion, organ transplantation, needle sharing and transplacentaly (so called congenital malaria) [44-46]. There are over 120 species of Plasmodium but today mainly five of these are known to infect humans: *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*. However, the main hosts of *P. knowlesi* are macaque apes in South-East Asia and the infection has in the last decade been recognised also as a cause of human malaria [47]. *P ovale* has recently been shown to actually consist of two different species; *P. ovale wallikeri* and *P. ovale curtisi*, with longer latency periods reported for the latter [48]. *P. falciparum* and *P. vivax* are the two species responsible for the greatest disease burden in humans. *P. vivax* has the widest global distribution, responsible for approximately 40% of the infections outside the African region [49], still causing occasional local infections in Southern Europe, and can lead to relapsing malaria due to a dormant liver stage. *P. falciparum* is most prevalent in Sub-Saharan Africa and is responsible for 99% of deaths [49]. The geographical distribution of malaria affected areas overlaps with the habitat of the *Anopheles* mosquito, but is also restricted to a climate suitable for the plasmodium species, i.e. tropical and sub-tropical areas. Previously there were locally acquired cases of malaria in Sweden, but with the use of quinine, improved housing, draining of marshes and generally improved socioeconomically standards, local transmission was eliminated around 1930 [50].

1.2.2 Life cycle

A malaria infection starts with the inoculation of *Plasmodium* sporozoites from the salivary glands of a biting female *Anopheles* mosquito into the blood-stream of a human, or into the subcutis, from where they trickle into the blood stream over a few hours, thereafter invading liver cells (hepatocytes) [51]. From early experimental studies in humans, there is evidence that the sporozoites are present in the blood circulation only up to 60 minutes after inoculation [52]. In the hepatocytes, sporozoites replicate asexually (so called exo-erythrocytic schizogony) and form pre-erythrocytic schizonts containing thousands of daughter merozoites. Once the schizonts have matured, they rupture and release the merozoites into the blood stream. This maturation process can take 6-21 days depending on species, and together with the following much shorter cycle of erythrocytic schizogony, it corresponds to the incubation time. Thus, this part of the infection is asymptomatic. Sporozoites of the *P. vivax* and *P. ovale* can develop into a dormant stage called hypnozoites

that can remain in this stage for months up to several years, with relapsing frequency influenced by transmission seasonality, before resuming schizogony and blood stage infection [53].

The released merozoites then invade the erythrocyte by a multiple step process involving the merozoite surface proteins (MSP), apical membrane antigens (AMA), erythrocyte binding antigens (EBA) and *P. falciparum* reticulocyte binding homologue proteins (PfRh) [54], several of these which have been vaccine candidates. Within the erythrocyte, asexual replication once again takes place (erythrocytic schizogony) and the merozoites develop into trophozoites (also called mature ring-forms, a stage easily identifiable in microscopy) and then to erythrocytic schizonts. Upon rupture, the erythrocyte releases 6-36 merozoites (*P. falciparum* schizonts containing the most) that then invade new red blood cells. This is the start of the clinical infection. The erythrocytic replication takes approximately 24 hours for *P. knowlesi*, 48 hours for *P. falciparum, P. vivax* and *P. ovale*, and 72 hours for *P. malariae*.

For the replication stages in both hepatocytes and erythrocytes, the parasite needs nutrients from their host. Upon invasion, the permeability across the human erythrocyte membrane is increased, so that important nutrients more easily can be taken up [55]. Increased flux of ions, aminoacids, lipids and certain vitamins have been observed [56], and several fold higher influx of glucose into infected erythrocyte than in uninfected cells [57].

At the same time as the parasite replicates, a complex protein machinery is developed in the erythrocyte and parasite derived surface protein are expressed on the erythrocytic surface. The most well studied of these are the *P. falciparum* erythrocyte membrane protein 1 (PfEMP-1) involved in pathogenicity of the parasite by mediating the binding of erythrocytes to the endothelial walls, resulting in sequestration of infected red blood cells in various organs and tissues [58]. Some of the merozoites released by red blood cells will develop into the sexual form of the parasite; gametocytes, that are ingested by feeding female *Anopheles* mosquitos. For *P. falciparum* this process usually starts after several rounds of asexual erythrocyte replication while for *P. vivax* gametocytes are formed soon after the release of merozoites from the liver. Thus *P. vivax* actually can transmit before symptomatic stages of the disease [59]. After ingestion, the gametocytes reproduce within the mosquito gut resulting in diploid zygotes that develop into ookinetes that migrate from the gut lumen to the gut wall where they develop into oocysts. The oocysts then rupture and release thousands of sporozoites that migrate to the salivary glands of the mosquito from where they once again can infect a human.

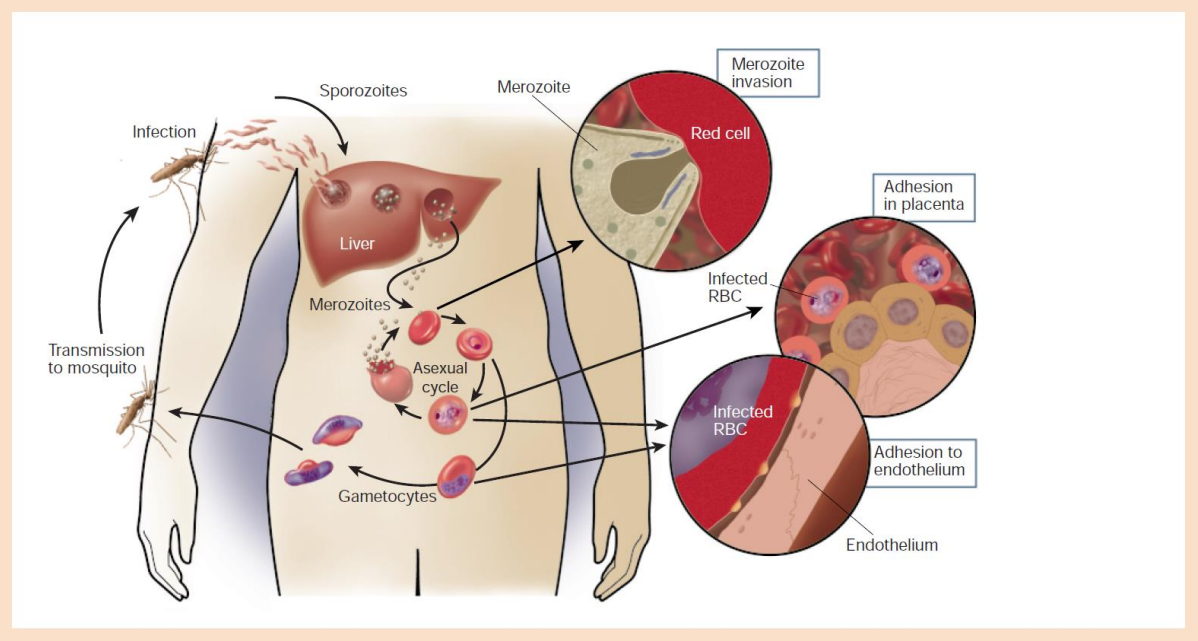

Figure 4: Plasmodium falciparum life cycle and pathogenesis
Source: Miller et al, "The pathogenic basis of malaria", Nature, 2002, Reproduced with permission from Springer Nature.

1.3 CLINICAL PRESENTATION

The first symptoms of malaria are often non-specific, imitating a flue; with fatigue, nausea, headache, muscle and joint pain, followed by high fever, chills, sweating sometimes accompanied by vomiting and diarrhoea. Symptoms differ somewhat in children and adults. Children usually present with less chills, arthralgia/myalgia, headache, more often GI symptoms, cough and non-specific symptoms like lethargy, malaise and poor feeding [60].

The symptoms of malaria are associated with the rupture of erythrocytes infected with shizonts which triggers a cascade of inflammatory responses initiating the classical symptoms of fevers and rigors. The fever is usually irregular but in an untreated *P. vivax* or *P. ovale* infection in semi-immune individuals the typically described periodicity can be seen with fever every 48 hours and every 72 hours in *P. malariae*, which is explained by synchronized erythrocytic replication [61].

In children living in endemic areas, repeated infections give rise to anaemia, both through hemolysis of erythrocytes and parasite induced bone-marrow depression, and with high parasite burden this anaemia can become very pronounced (see severe malaria) (Perkins 2011). Adults living in areas with high transmission generally develop protection against clinical malaria and infections only occasionally give rise to symptoms, presenting then as a flue like illness. In these areas more than 50% of a population can have asymptomatic parasitaemia [62], with lower prevalence in areas with lower endemicity [63]. Duration of an asymptomatic infection depends on both transmission intensity [64] and age [65]. As an example, median durations of 9-60 days was reported among children in Papua New Guinea

[66]. In strictly seasonal settings, longer duration of infections over serval months of the dry season is a prerequisite for transmission the following year. However, duration of asymptomatic infections is not well explored and there are case-reports describing adult immigrants living in malaria-free countries with chronic *P. falciparum* infections persisting for longer periods; in one case for 13 years [67].

1.3.1 Severe *P. falciparum* malaria

P. falciparum infections can, depending on the immune status of the individual, progress to severe and potentially fatal disease. Severe malaria classically manifests as one or several complications indicating organ dysfunction including unrousable coma, metabolic acidosis, respiratory distress, severe anaemia, hypoglycaemia, acute renal failure, circulatory chock and hyperbilirubinaemia (for complete description of criteria see "Definition of severe malaria"), but there are great variations in the presentations of severe symptoms depending on age [68].

In children in endemic settings, severe malaria typically presents as three different but often overlapping syndromes: cerebral malaria, severe anaemia and respiratory distress (as a clinical proxy for metabolic acidosis), with the highest mortality reported in children with both respiratory distress and impaired consciousness (32%) [69]. The manifestations of severe malaria have different age patterns and may depend on transmission intensity [70]. Severe anaemia is the most common manifestation in children <2 years of age in areas of very intense transmission [71, 72], with a few studies showing a lower incidence in areas with lower transmission [71, 73]. In contrast, a larger proportion of children with severe malaria have cerebral malaria in areas with lower transmission compared to areas with the highest transmission, and peak incidences are seen at 3-5 years of age in moderate transmission areas [71, 73, 74]. In a hospital based study from Kenya it was demonstrated that with declining malaria transmission, the mean age of the children admitted increased, as did the ratio of cerebral malaria compared to severe anaemia [4]. In areas with low or epidemic transmission and in non-immune travellers, cerebral malaria is seen across all age groups, as well as other manifestations of severe malaria such as pulmonary oedema, circulatory chock and renal failure. [75-77]. The manifestations reported in different clinical settings may however also be affected by health care standard and diagnostic possibilities. Thus as medical diagnostics in general have improved, a recent study reported that kidney failure among African children with severe malaria was much more common than previously described, and also significantly associated to fatality [78].

Untreated, severe *falciparum* malaria leads to death in the majority of cases [68], but even with adequate treatment severe malaria carries high mortality in both children and adults; 8-24% in low-resource settings [79-81] and 10-15% in high-resource settings [77, 82-84].

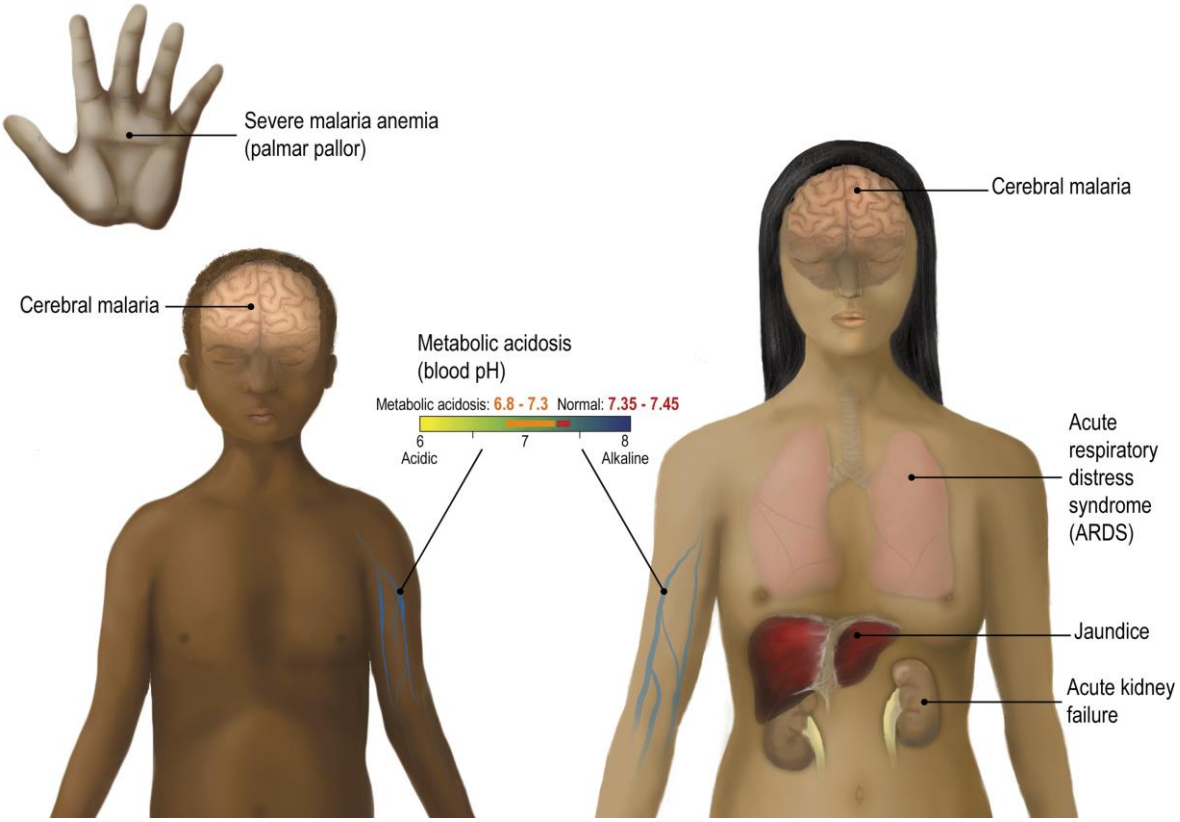

Figure 4: The major clinical complication associated with adult and paediatric severe malaria. *Source: Wassmer et al "Investigating the Pathogenesis of Severe Malaria: A Multidisciplinary and Cross-Geographical Approach", American Journal of Tropical Medicine and Hygiene (AJTMH), 2015. Reproduced with permission from AJTMH.*

The intervention with best evidence to reduce malaria mortality in patients with severe malaria has been the introduction of intravenous treatment with artesunate [85]. Despite this, artesunate availability is limited in some countries, and in the United States quinine was just recently replaced by artesunate as first line recommended treatment for severe malaria [86]. Randomized clinical trials evaluating other interventions for patients with severe malaria are sparse [87], and there is much left to be optimized in the management of patients with severe malaria, in both low and high resource settings.

1.3.2 Severe non-*falciparum* malaria

Although previously regarded as unusual, severe malaria can also develop in infections with non-*falciparum* species. Several reports, mainly from endemic settings, have observed severe anaemia, thrombocytopenia, liver dysfunction, kidney failure and splenic rupture in *P. vivax* malaria [88-90], but lately also cases with the classical syndromes of severe *P. falciparum* have been reported; such as cerebral malaria, shock and acidosis [91-93], possibly due to the increased virulence of certain strains [94]. In Indonesia, the prevalence of severe malaria was actually observed to be higher among patients with *P. vivax* compared to *P. falciparum*; 23% presented with severe disease, with severe anaemia as the main complication [88]. *P. knowlesi* has in recent years also been associated with high parasite densities and similar severe syndromes as for *P. falciparum* [95, 96], including death [47]. Severe manifestations

in *P. malariae* and *P. ovale* have only recently gained attention [97-100], but possibly severe cases are underdiagnosed both because of misdiagnosed species and since clinical and laboratory parameters included in the severe criteria are not as rigorously controlled. Except for occasional case reports [101-103], and a few observational studies based on notified data [104, 105], studies of severe malaria in Europe focus on *P. falciparum*. Risk factors for severe non-*falciparum* malaria have not been systematically evaluated in a non-endemic setting.

1.4 PATHOGENESIS OF SEVERE MALARIA IN *P. FALCIPARUM*

Pathogenesis in severe malaria is not yet fully understood but several discoveries specific for the biology of *P. falciparum* has led to the proposal of following mechanisms: 1) High multiplication rate as well as ability to infect erythrocyte of all ages (in comparison to *P. vivax* that prefers young reticulocytes) leading to high percentage of infected erythrocytes [106] 2) Sequestration, i.e. adhesion of infected erythrocytes to endothelial cells in the capillaries which protects infected red blood cells from being cleared by the spleen [107], results in reduced blood flow leading to tissue hypoperfusion, anaerobic metabolism and metabolic acidosis [108]. Studies have shown that this sequestration is not uniformly distributed but more pronounced in certain organs such as the brain [109]. Sequestration is also involved in the pathogenesis of placental malaria [110]. 3) Reduced deformeability of both infected and uninfected erythrocytes which leads to additional obstruction of blood flow in the microvasculature [111] 4) Rosetting; a phenomenon involving the binding of uninfected erythrocytes to *P. falciparum* infected erythrocytes [112]. 5) Excess production of pro-inflammatory cytokines (TNF-α, IFN-γ, IL-1) and possible also the release of mediators such as nitrogen oxygen [113].

Both sequestration and rosetting involves special surface proteins, so called adhesins, most importantly the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), that the erythrocyte expresses when infected by *P. falciparum* and which both can bind to other erythrocytes or epithelial cells [114]. A newly published study has identified certain kinases, only found in *P. falciparum* that control “stickiness” of red blood cells [115].

In addition, there is evidence that there are specific strains genotypes of *Plasmodium* that are more prone to cause severe malaria infection [116].

1.5 DEFINITION OF SEVERE MALARIA

Severe malaria is defined by clinical or laboratory evidence of vital organ dysfunction. Strict definitions of severe malaria were initially designed by WHO to identify children with severe malaria in high endemic and often poor resource settings, but also to be used for epidemiological and research purposes. Definitions and guidelines on management of severe

malaria were first published by WHO in 1986 and since then, several updates have been published with slight changes of the criteria for every new version. Hyperparasitaemia was initially included as a criteria, with different cut-offs depending on patient immunity status [68]. Lately it has been questioned if hyperparasitaemia should be viewed as a risk factor for severity rather than an indicator of severe malaria when unaccompanied by other indicators of severe diseases, and in the 2012 WHO definition it was not included as a single criterion [117]. In the most recent WHO publication hyperparasitaemia is again an independent criteria, with a threshold of >10% irrespective of previous exposure, as well as definitions for severe *P. vivax* and *P. knowlesii*, however without the parasitaemia criteria [41].

Several studies have tried to pin-point which criteria are the most prognostic for predicting fatal outcome in children and adults, and different scores have been developed as an aid; among others the Malaria Severity Assessment (MSA) and Coma Acidosis Malaria score (CAM) [118, 119]. The strongest predictors for mortality in both children and adults have repeatedly been identified as coma and acidosis, alternatively respiratory distress as an indirect measure of acidosis, but also kidney failure has been shown to be an important predictor in those settings that allow for assessment of renal function [69, 81, 118, 119].

In a clinical context, the severe malaria criteria are probably too strictly defined and any sign of vital organ dysfunction should lead to increased surveillance and readiness to start parental treatment and intensive care.

1.6 DIAGNOSTICS AND TREATMENT

1.6.1 Diagnosis

1.6.1.1 Microscopy

The gold standard for diagnosing malaria is by microscopy, as it enables both species determination and parasite quantification. Thick and thin blood films are stained with either Giemsa or Field's stain and examined by microscopy. If parasites are detected in thick smear, parasite count and species detection are usually done in thin films, although in some settings parasite count is estimated in thick smear by counting parasites either against leukocytes or ocular fields. In thick film, the lower limit of detection of parasites is 1 parasite in 200 ocular field (approximately 5-10 per microliter),but depending on the experience of the microscopist.

1.6.1.2 Diagnostic Rapid Tests

Malaria rapid diagnostic tests (RDTs) detect Plasmodium antigens by antibody-antigen interactions on a test strip. Histidine Rich Protein 2 (HRP 2) is the most commonly used antigen specific for *P. falciparum*, while Pan LDH (lactate dehydrogenase) can be used for detecting both *P. falciparum* and non-*falciparum* species. Most RDTs used in Sweden contain both antigens, thus detecting *P. falciparum* as well as non-*falciparum* (*P. vivax, P.*

ovale, P. malariae). One widely used RDT in Sweden (CareStart™ Malaria Combo Test) can detect down to 50 parasites per microliter in *P. falciparum* infections. Sensitivity is good for *P. falciparum* and *P. vivax* (but depending on the level of parasitaemia), however for *P. ovale* and *P. malaria* sensitivities of around 30% have been reported [120].

In the Swedish setting, rapid diagnostic tests are considered a supportive diagnostic tool and are only used as a complement to microscopy, aiding the acute diagnostics outside office hours. Both positive and negative rapid tests need to be confirmed by microscopy. In positive tests, microscopy is needed to assess species and level of parasitaemia since high parasitaemia is a criterion for severe malaria and requires intravenous treatment. Negative rapid tests also need to be controlled by microscopy since false negative tests may occur, either due to the so called prozone effect (excess of antigens binding to the detection antibodies) in high parasitaemic infections with *P. falciparum*, or due to HRP2 negative strains of *P. falciparum* that have become increasingly detected in some areas of the world, such as Eritrea, Rwanda and the Amazon [121-123].

1.6.1.3 Molecular methods

The detection of *Plasmodium* species DNA by polymerase chain reaction (PCR) is the most sensitive method of parasite detection and determination of species but is seldom used in the acute clinical setting, since it is not as easily available, more expensive, and takes longer to analyze than microscopy. With PCR, normally down to 1 parasite/microliter can be detected, but lower levels are possible to detect if larger blood samples and high sensitivity PCR is used [124]. PCR can remain positive for weeks after the *Plasmodium* infection has been successfully treated (up to 42 days has been reported in travellers) [125], and microscopy is therefore needed to confirm an ongoing infection after treatment.

Another more recently developed molecular method, loop-mediated isothermal amplification (LAMP) with similar sensitivity to PCR [126], that amplifies nucleic acid under isothermal conditions, which means that the expensive thermocyclers used in PCR are not needed, making the method suitable also for parasite detection and species determination in low-income countries. LAMP has only been routinely used in one region of Sweden, and is planned to be implemented for acute diagnostics at the malaria reference laboratory in Stockholm.

1.6.2 Treatment

For uncomplicated *P. falciparum* and *P. knowlesi* malaria, the recommended treatment is artemisinin-based combination therapy (ACT) [41]. Uncomplicated *P. vivax, P. ovale* and *P. malariae* infections can usually be treated with chloroquine, but there are certain areas with chloroquine resistant *P. vivax*, where treatment with ACT is preferred. In *P. viva*x and *P. ovale* an additional two week course of primaquine is given to eradicate hypnozoites, which

are not killed by neither ACTs not chloroquine and otherwise will be left in the liver with potential to relapse.

In complicated malaria, irrespective of species, and in patients that are vomiting, intravenous artesunate is now the WHO recommended first-line treatment, also for pregnant in all trimesters, due to its very high parasite clearing time compared to quinine [85]. However, if there is no artesunate available, quinine administered intravenously is given instead.

1.7 MALARIA AND IMMUNITY

1.7.1 Natural acquisition of immunity

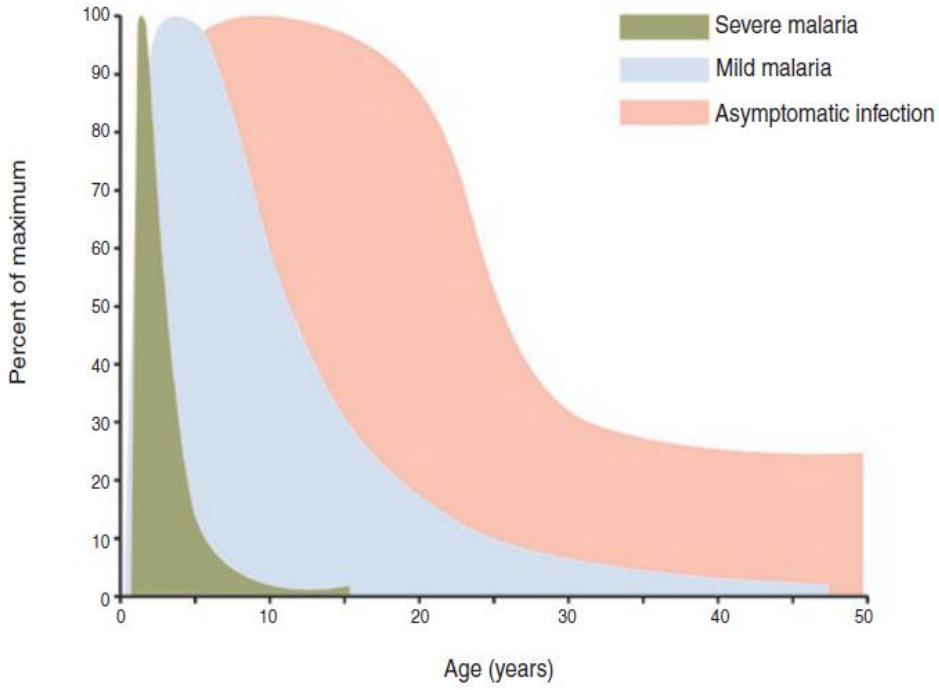

Figure 5. Schematic representation of incidence of severe, mild and asymptomatic malaria in relation to age, in an area with high *P. falciparum* transmission. *Source: Langhorne et al, "Immunity to malaria: more questions than answers", Nature Immunology, 2008. Reproduced with permission from Nature Publishing group.*

Development of immunity to malaria is complex and there are still many knowledge gaps to fill. In the 1920s malaria was deliberately induced in neurosyphilis patients as a treatment, and during these experiments it became evident that immunity to malaria develops gradually in individuals that are repeatedly infected [127]. Malaria-antibodies seem to play an important role in controlling the infection since experiments from the 60s has shown that passively transferred IgG from semi-immune adults could reduce and sometimes clear parasitaemia in children with acute *P. falciparum* infections [128]. Characteristically, malaria immunity is not sterilizing, instead the host learns to control the infection and develops

protection first mainly against severe forms of malaria, eventually also against milder clinical symptoms. With a high degree of immunity, an individual can be continuously carrying parasites at low concentrations without any symptoms, but also seems to be protected against new infections, a state referred to as "premunition" [129, 130]. Immunity to severe disease is acquired faster than to mild disease, while protection reducing parasite load takes even longer to acquire [131]. Thus, in areas with high and stable transmission, severe malaria is mainly seen among small children [71, 131, 132], but rarely in infants that are proposedly protected by fetal haemoglobin [133] and maternal IgG transferred over the placenta [134]. Immunity against cerebral malaria has been proposed to develop after just 1-2 infections [135], however recent studies have indicated that one infection is not sufficient to protect against severe disease [136]. Mild infections are seen throughout childhood but in adolescence most individuals in holoendemic settings have developed the ability to control parasite densities and instead present with persistent asymptomatic infections [131]. Thus, in many parts of Sub-Saharan Africa, with stable malaria transmission, malaria could actually be viewed more as a chronic infection than an acute. An exception are pregnant women who loose clinical protection and present with both mild and severe manifestations as well as placental malaria [58]. The number of infections required to develop immunity is not yet established, however there are studies indicating that the process is more rapid in adults compared to children [137, 138].

1.7.2 Maintenance of immunity

Previously, clinical immunity to malaria has been thought to be lost within a few months in the absence of exposure [139], but there are studies indicating that the protection might be more long-lived. In Madagascar, where malaria re-emerged after efficacious control for 30 years, older adults who had lived during the period when there was continuous transmission, were less likely to develop clinical malaria than younger, previous unexposed individuals [140].

Results from a recent immunological study of Swedish travellers, indicate that there is a long-lived immunological memory towards malaria; as memory B-cells (MBCs) towards different malaria antigens were found in travellers up to 16 years after one single infection [141]. However, malaria specific MBCs are not found in all exposed individuals [141, 142] and the functional role of these MBCs is not entirely known. Higher antibody levels have been measured after an episode of acute malaria in immigrants compared to non-immune travellers supporting that there is an immunological memory that can be activated upon a new infection [143, 144].

Just as with acquisition of immunity, there are probably different mechanisms for maintenance depending on type of immunity: Immunity controlling parasitaemia seem to need persistent infection resulting in high levels of circulating antibodies that protect against symptomatic infection while immunity to severe disease might be maintained for longer periods even in absence of boosting [130]. This is supported by the majority of observations for imported malaria, where immigrants in non-endemic countries who previously were

highly exposed for malaria during their childhood presented with clinical malaria when re-visiting their home-country, however usually with milder symptoms and less often severe and deadly disease [145-149]. Bouchaud et al also described lower parasite densities and faster fever and parasite clearance in African immigrants who had lived in France for a median of 14 years, compared to European travelers. Although the majority had visited their home-country after immigrating to Europe, there was no difference in disease severity depending on frequency of prior visits to country of origin, implying that boosting of immunity was not a major contribution to the protection observed [143].

How long immunity to severe malaria may be maintained is debated. With the declining transmission seen in many endemic areas today, fewer individuals may develop protection against severe disease, possibly there is also a risk of loss of protection in individuals who previously have acquired immunity [150]. Maintenance of immunity is difficult to assess since populations in malaria-endemic regions are constantly exposed to new infections. but studies in travellers and immigrants with imported malaria can contribute with valuable information.

1.8 FACTORS AFFECTING OUTCOME

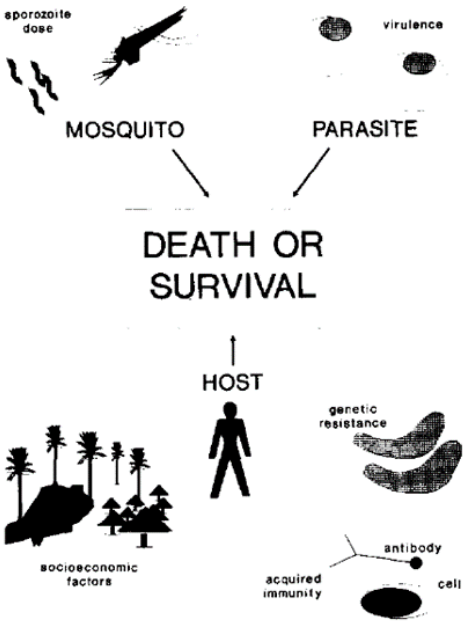

Figure 6. Factors with potential to affect outcome in malaria infections. *Source: Greenwood et al, "Why do some African children develop severe malaria?", Parasitology Today, 1991. Reproduced with permission from Elsevier*

Not all individuals with *P. falciparum* develop severe disease. The outcome of infection is a combination of several factors attributable to: 1) The parasite (such as genetic polymorphism, antigenic variation, rosetting, cytoadherence, drug resistance). 2) Sociogeography (such as

malaria transmission intensity, health care access, cultural, economic and educational factors) and 3) Inherited and acquired factors of the host [58]. Identification of factors associated with severity of disease is important in order to improve clinical guidelines for the management of malaria. One of the main aims of this thesis is to identify risk factors for severe malaria in travellers. Previously studied host factors in both endemic and non-endemic settings are introduced below.

1.8.1 Inherited host factors

Genetic traits in the host, most importantly sickle cell traits, but also thalassemias, blood group 0 and G-6PD-deficiency, have been shown to protect against clinical and/or severe *P. falciparum* malaria [151]. Lack of Duffy blood group antigen, a trait common in populations of West Africa, protects against infection with *P. vivax* since this species is partly dependent on the Duffy antigen receptor for invading erythrocytes [152].

Protection against severe malaria has also been associated with polymorphisms in the CD 36, ACE and ACE-2 genes [153, 154]. CD36 is a transmembrane glycoprotein involved in a variety of functions depending on where it is expressed, but it is also a receptor for *P. falciparum* infected red blood cells and has been reported to be involved in the sequestration of parasite erythrocytes [155, 156]. ACE and ACE-2 regulates angiotensin levels that among other functions has an impairing effect on the erythrocytic cycle. Interestingly, CD36 deficiency is also related with the metabolic syndrome [157, 158], and ACE polymorphism to hypertension [159].

1.8.2 Pregnancy

Malaria in pregnancy is a major contributor of global maternal and neonatal mortality and morbidity [160]. Pregnancy puts women at risk of clinical malaria with higher parasite densities, maternal anaemia, severe manifestations such as cerebral malaria and pulmonary oedema. Moreover, placental malaria is a risk factor for both severe anaemia in the mother and low birth weight and mortality in the infant [15, 161]. While pregnant women in high-transmission areas can have clinically silent infections but often with placental engagement and anaemia, women in low-transmission settings are at major risk of severe manifestations and fatal malaria [160], with reports of a three-folded risk of severe malaria [76, 162]. There is less data from non-endemic settings, but occasional severe and fatal cases of imported malaria in both semi-immune and non-immune pregnant women have been reported [77, 163, 164]. A recent pooled analysis of 631 pregnant women with imported malaria diagnosed in eight different sites, reported no deaths, but 16% (46 of 285 where data on maternal outcome was available) with severe malaria and nearly 50% of 95 cases with available data on offspring outcome presented with offspring complications [165].

1.8.3 Comorbidity

Several coinfections have been shown to affect the disease course and outcome of malaria. HIV infection is a well-known risk factor, that in numerous studies has been associated to

both severe disease and mortality of malaria, especially in patients without previous immunity, pregnant women, and in HIV positive patients with low CD4 count [18, 166, 167] Tuberculosis infection has been demonstrated to modulate the host response to malaria [17] and there are a few reports with contradicting results of chronic hepatitis´ effect on malaria disease course in populations with different immunity [168-170]. Splenectomy has also been observed to affect disease course [171] and in a study of severe imported malaria to France 7.3% of patients had some kind of immune deficiency [77]. However, the role of immunodeficiency disorders other than HIV and the potential effect of immunosuppressive drugs have not yet been systematically evaluated, despite the increasing use of these medications, also in travellers to malaria-endemic countries [172].

The role of non-communicable diseases (NCD) has previously only been assessed in a few studies. Two studies from Ghana have shown that semi-immune adults with type 2 diabetes were more susceptible to *Plasmodium* infection than controls, but there was no inference on severity [173, 174]. Overweight was associated with progression to severe malaria after treatment start in uncomplicated cases in Thailand [175], and in another report from Thailand, fatal cases had higher BMI compared to non-fatal severe cases [176]. Considering that both diabetes and obesity have been shown to affect disease severity in other infections [177], and that NCDs are increasing also in areas with high malaria transmission [178], there was a need for investigating how these comorbidities might affect severity of malaria.

1.8.4 Risk factors previously studied in travellers

Imported malaria requires special attention because of the potentially high virulence of the infection in non-immune travellers and the risk of delayed diagnosis due to limited awareness among clinicians. In this PhD-project, the term “travellers” also refers to immigrants from endemic areas visiting friends and relatives, as well as newly arrived immigrants and temporary visitors from regions with malaria transmission.

The overall case fatality rate in travellers with imported malaria varies between 0.3-4% depending on site of diagnosis, proportion of *P. falciparum* cases, and constitution of the study population, most importantly regarding patient origin [82, 145, 179-182]. Approximately 4-10% of *P. falciparum* infections in travellers present as severe malaria [83, 183, 184]. Despite good surveillance system in Sweden, neither case fatality rate nor proportion of severe cases is known in Sweden since the surveillance data provided by the Public Health Agency does not include data on outcome and clinical symptoms.

1.8.4.1 Age

Age is one of the most important host factors affecting malaria presentation, with different implications depending on level of transmission. As described earlier, in endemic settings, those at highest risk of severe malaria and death are children under 5 years of age, although reports based on verbal autopsies from endemic areas indicate there can be a substantial numbers of deaths due to malaria also in the elders [185]. In low endemic settings such as Southeast Asia, fatality increased stepwise from 6% in children <10 years to 36% in adults

aged >50 years of age [186]. Among non-immune travellers, age has been shown to be one of the most important independent risk factors for both severe malaria [83, 183, 187, 188] and fatality [82, 145, 146]. Checkley et al observed a 10 folded increased odds of death in malaria for individuals >65 [145], but the risk of death increases already after 50 years, with reports of approximately 80% risk increase for each decade [82].

With increasing age, comorbidities such as hypertension, diabetes and cardiovascular events become more common and an increasing proportion of travellers today belong to an elderly population, often affected by one or several chronic conditions [189, 190]. In a large French study of severe falciparum cases 14% had at least one other comorbidity [77] and in a European multi-center study 43% of severe cases had other underlying comorbidities reported, hypertension and HIV being the most common [191]. However, there have previously not been any studies that have systematically assessed if and to what extent pre-existing comorbidities could explain the effect of age on severity.

1.8.4.2 Patient origin

With few exceptions [192, 193], repeated studies have reported both significantly increased case fatality rates and a higher proportion of severe malaria among travellers from non-endemic countries compared to individuals with endemic origin, with up to an eight folded increased risk for both severe malaria and death [82, 143, 147, 148, 179, 188, 194]. The great differences have mostly been attributed to previous exposure of malaria and corresponding semi-immunity as discussed earlier.

However, country of origin can only serve as a rough proxy for categorizing patients as non-immune or semi-immune, and other modes of classification have also been proposed, such as self-reported previous exposure or ethnicity. Two studies reported a five to eight times increased odds of severe malaria in white individuals, and Philips et. al. could also show that the effect of ethnicity was independent of previous exposure [188, 195]. In contrast, although a higher proportion of fatal cases were classified as white in a large French study of severe malaria (n=400), ethnicity could not be associated to in-hospital mortality after adjustment for other host factors [77]. Possibly, part of the reduced risk for severity observed in populations with certain origin and ethnicity can be explained by inherited traits such as thalassemia or sickle cell anaemias that are seldom examined in epidemiological studies, but despite the more common prevalence of these traits in African populations, they could only explain a few percentages of the total protective effect [196].

As has been discussed previously, both time lived in an endemic area and duration of residency in a malaria free country will probably influence the level of immunity in an individual with endemic origin, but these factors are rarely taken into account. To assess the effect of a potential long-term immunity on clinical presentation of malaria, a few studies have excluded newly arrived visitors or immigrants with short term residency in non-endemic areas: One study reported 4% severe malaria among immigrants of endemic origin who had lived in Europe for at least 4 years compared to 15% in European travellers [143]. Similarly,

another study reported 3% severe malaria among African travellers with at least one year of stay in a malaria-free country compared to 11% in travellers of European origin [149]. One study performed over different periods reported severe malaria only among immigrants residing for 13–19 years in Italy [187]. However, until now, no study of imported malaria has conducted a time-specific analysis of severity in relation to years spent in non-endemic country, thus the duration during which protection against severe malaria is maintained is unknown.

1.8.4.3 Reason to travel

Travellers visiting friends and relatives (VFR) have been observed to be at lower risk of severe and fatal malaria compared to "tourists" [145, 149, 197]. Although the term VFR most often corresponds to those individuals born in an endemic country, and thus indicates previous exposure, the definition varies in-between studies, and sometimes also includes travellers born and grown up in a non-endemic country but with parents or relatives from endemic areas. A few studies have tried to separate the terms and have assessed disease outcome in relation to both patient origin and reason for travel [145, 192]. In a large UK study of imported malaria, a lower case fatality rate was observed in VFR compared to tourists also when restricting the comparison to only travellers with endemic origin, suggesting that there are additional factors affecting outcome unrelated to patient origin, such as different disease awareness and health seeking behavior [145].

1.8.4.4 Chemoprophylactic use

Inadequately or absence of chemoprophylaxis intake has in several studies been reported to affect disease outcome in travellers, especially in patients with non-endemic origin [82, 148, 181, 183, 198]. Probably, chemoprophylaxis intake that fails to protect an individual from infection can still succeed in lowering parasitaemia and thus both risk of severe disease and fatality. Nevertheless, a problem with assessing this factor is that it is entirely based on patient report (and might be associated with recall bias). A consequent of chemoprophylaxis that is important for clinicians to be aware of, is the possibility in delayed onset, which can complicate diagnostics [199].

1.8.4.5 Gender and sex

In the majority of epidemiological studies over imported malaria, a larger proportion of cases are seen in male [145, 147, 180, 194, 200]. Behavioral factors have been proposed as the main explanation for this difference, for example risk taking, medical health seeking, use of prophylaxis and other protective measures. Still, to some extent there could be a biological component; such as the production of olfactory cues that make men more attractive than women for malaria transmitting mosquitoes [201], and possibly immunological, as has been shown for other infectious diseases [202]. One Italian study observed a higher ratio of females among severe cases [197] in contrast to two large independent studies from France that reported a higher ratio of males among fatal cases, however, in multivariable analysis, gender was not significantly associated to death in neither study [77, 82], and no other study

has been able to show an independent association between gender and fatal outcome. Among 22 029 malaria patients hospitalized in the USA 2000-2014, similar proportion of uncomplicated and severe cases were reported in males and females. Even so, a larger proportion of men had severe manifestations in terms of renal failure and jaundice, while severe anaemia was more common among women (also in non-pregnant) [203].

1.8.4.6 Treatment delay

Both due to delay in seeking care (patient delay) or delayed diagnosis (health care or doctors delay) has in several studies been observed to be associated to higher fatality [84, 145, 181], as well as to severity [83]. In one study a prior visit to a general practitioner also was independently associated to severe disease [83] and in a large study from UK, the case fatality rate was affected by the number of malaria cases seen in a region, with an adjusted OR of 18 for death if the diagnose was made in a region where malaria presented infrequently [145]. Earlier studies have also reported suboptimal treatment of patients with imported malaria presenting at health care centers without expertise in tropical medicine [184].

1.9 MALARIA AND LONG-TERM EFFECTS

Although malaria is well recognized to be a potentially severe and fatal disease, it has until recently not been regarded to have any long-term effects on health when the infection has been cured. There is now however a well-established associated between malaria and Burkitt's lymphoma in African children [204] and growing evidence of post-malaria neuro-cognitive impairment in children [13, 205, 206].

Other potential long-term consequences have not been systematically assessed, and little is known about long term consequences of malaria in adults and in travellers. Identifying long-term effects of malaria is important for adequate assessment of the global burden of malaria and associated DALYs.

1.9.1 Malaria and cancer

Several infectious agents, both viruses, bacteria and parasites, have been shown to have oncogenic effects in humans and an estimated 20% of all cancers globally have been associated to infections [207].

Malaria has been proposed to have both anti-tumour and oncogenic capacities. In 18th and 19th century malaria was believed to have cancer protective effects based on observations that cancer increased in Europe after decrease in malaria incidence and there have even been earlier clinical trials evaluating malaria therapy in terminal cancer patients [208]. Later mouse model experiments suggested that malaria infection could inhibit growth in solid cancers [209]. In a recent ecological study, an inverse correlation between cancer mortality and malaria incidence was described, however only 29 cancers were analysed and data for several

countries especially in Africa lacking, and most importantly, cancer mortality does not necessarily correlate to cancer incidence [210].

In 1962, Denis Burkitt described a strong geographical association between holoendemic malaria and a common tumour seen in African children that later was classified as a non-Hodgkins lymphoma; endemic Burkitt lymphoma (eBL) [211]. A few years later, Michael Anthon Epstein demonstrated that multiple copies genome from the Epstein-Barr virus (EBV) was present in nearly all eBL tumour cells [212] and EBV infection was later demonstrated to be capable of transforming B cells to produce polyclonal tumours. However, in immunocompetent individuals, most EBV infections do not lead to cancer development and EBV is not restricted to Sub-Saharan Africa.

Since then multiple studies have provided evidence for the etiological role of *P. falciparum* in the development of eBL [213-215]. Epstein–Barr virus (EBV) has been demonstrated to be a necessary cofactor since multiple copies of viral genome are present in nearly all eBL tumour cells and EBV infection is capable of transforming B cells to produce polyclonal tumours [204].

Figure 7. Child with suspected Burkitt lymphoma, *Peadiatric ward, Muhimbili University Hospital, Dar es Salam, Tanzania. Photo: Katja Wyss, with permission from child's mother*

There are reports suggesting that malaria could predispose also to other Non-Hodgkins lymphomas [216, 217], as well as to leukemias [218], but further studies to confirm these observations are lacking. Moreover, it is speculated that malaria could be associated to other cancer forms [219], and higher incidence rates of cervical cancer (another virus induced cancer) were observed in areas with high malaria endemicity in Uganda [220].

Malarias potential carcinogenic role in humans clearly need to be further investigated, and until now, studies with longitudinal design assessing the risk of cancers in adults with previous malaria have not been published.

1.9.2 Malaria and neurocognitive sequelae

Several studies have reported both neurological [12, 205, 221] and cognitive impairments [222-224], following cerebral malaria in children in endemic areas and it has been estimated that 24% of children with cerebral malaria develop some kind of neurocognitive sequelae [225]. Both epilepsy and neuro-disabilities have been reported following cerebral malaria,

with persistent cortical blindness frequently observed [221, 225, 226]. All categories of cognition have reported to be affected; most importantly working memory but also attention, language, visuospatial skills and executive function [206, 222]. Lately, an increasing number of studies have demonstrated long-term behavioral and mental health problems in children, including ADHD-like disorders [227-229]. There are also a few reports demonstrating that episodes of severe non-cerebral malaria [223, 230] and even uncomplicated malaria can affect cognition, behavior and school performance in children [231-233].

Neurological impairment in adults has been sparsely studied. In a large prospective Vietnamese study transient neuropsychiatric disorders referred to as "post-malaria neurological syndrome" were observed after both uncomplicated and severe falciparum infection [234]. In contrary, two other reports have demonstrated permanent neuro-cognitive and psychiatric sequelae many years following cerebral malaria [235, 236]. A case-series study of imported malaria reported neuro-cognitive sequelae, in particular memory impairment, in six adult travellers with severe malaria, however follow-up was only 6 months [237]. No studies have yet systematically assessed long-term neuro-cognitive and behaviour sequelae, following malaria infection in adult travellers.

1.9.3 Malaria and other long-term effects

Severe malaria is well known to cause acute complications in other organs than the brain, [68] and in line with the pathogenesis of long term neuro-cognitive effects after cerebral malaria, chronic effects in other organs would be expected. *P. malariae* has been demonstrated to cause nephrotic syndrome through immune complex deposition in the kidneys, and in some cases the renal disease becomes chronic [238]. The predominant histopathology found in the acute kidney injury related to severe Plasmodium falciparum is ischemic acute tubular necrosis, and this kidney injury has previously been stated to be reversible in survivors [68, 239] However studies of other infectious diseases have shown that there is a long term risk of renal complications in patients with acute kidney dysfunction, even in those patients that fully recovered baseline kidney function at dismissal [240]. The use of WHO criteria for evaluation of acute kidney injury (AKI) and possible chronic complications is questionable because of high creatinine cut-off value (>265 μmol/L) and disregard of previous kidney function. There are now more recent reports assessing AKI also using the criteria set by KDIGO (Kidney Disease: Improving Global Outcomes) where the dynamic of GFR is taken into account. These studies have observed other histopatholgies involved in acute kidney injury and also reported long-term effects including irreversible renal disease [241, 242].

In addition to potential effects on individual organs, malaria has recently been shown to shorten the lifespan in birds by telomere degradation [243]. Other infectious diseases have been reported to affect biological ageing in humans [244, 245]. Results from our research

group indicate that transient shortening of telomeres also occurs in travellers with malaria, but we lack data concerning potential effect on life span [246].

Long-term health consequences of malaria are difficult to study in endemic settings where reliable health and recording systems often are deficient, and the diagnosis of clinical malaria is complicated by asymptomatic infections. Register based studies in travellers, where diagnosis is ascertained and follow up over several decades possible, can clearly be beneficial for the assessment of malaria-related effects on health and survival.

2 AIMS

The overall aim of this thesis was to identify risk factors for severe malaria and long-term consequences from malaria in travellers and migrants, with the intent to improve the acute and follow-up management of malaria.

2.1 SPECIFIC AIMS

- To describe the clinical presentation and outcome of imported malaria in Sweden (Study I-III)
- To assess whether duration of residency in a malaria-free country affects the risk of severe *Plasmodium falciparum* malaria in immigrants from Sub-Saharan Africa (Study I)
- To assess if comorbidity, in terms of chronic diseases and obesity, is associated with severe *P. falciparum* malaria (Study II)
- To assess factors associated with disease severity in *P. falciparum* and non-*falciparum* malaria (Study III)
- To investigate if patients with malaria have an increased risk of developing lymphoid neoplasms and cancer overall (Study IV)

3 METHODS

This section presents the setting and summarizes the methodology and epidemiological terminology for the studies included in this thesis. A more thorough description is presented in the method section of the respective papers.

3.1 OVERVIEW

All studies (I-IV) included patients diagnosed with malaria in Sweden, identified through the National Surveillance system at the Public Health Agency of Sweden as well as additional cases provided by diagnosing hospitals and microbiological departments that had not been notified to the Public Health Agency. In study III multiple episodes were included, in the remaining studies we only included first episode of malaria reported in an individual. Case definition included microbiological confirmed episodes only, in most cases through detection of *Plasmodium* in blood by microscopy, or, in rare cases, detection of nucleic acid from *Plasmodium* species by PCR. In study IV patients were also identified by discharge diagnoses in the National Inpatient Register or diagnoses registered at outpatient visits in the National Outpatient Register, in these cases the malaria diagnoses were not microbiological confirmed.

Table 1. Overview of methods for study I-IV

Methodology for study I-IV				
	Study I	**Study II**	**Study III**	**Study IV**
Study design	Cross-sectional	Cross-sectional	Cross-sectional	Cohort
Study population	Adults diagnosed with *P. falciparum* in Stockholm	Adults diagnosed with *P. falciparum* at 18 hospitals Sweden	Adults and children diagnosed with malaria (all species) of malaria in Sweden	Adults and children with complete PIN, diagnosed with malaria (all species)
Time period	1995-2013	1995-may2015	1995-2015	1987-2015
Number	501 patients	937 patients	2653 episodes	4125 patients
Identification of malaria cases	Public Health Agency of Sweden	Public Health Agency of Sweden	Public Health Agency of Sweden	Public Health Agency of Sweden + NPR
Handling of multiple episodes	Only first episode included in analysis	Only first episode included in analysis	All episodes (except relapses and recrudescences)	First confirmed episode included, multiple episodes analysed separate
Data sources	Medical records	Medical records	Medical records	Cancer Register, Cause of Death Register, Total Population Register, Medical records
Main statistical methods	Nonparametric tests + logistic regression	Logistic regression, multiple imputation	Logistic regression	Cox regression
Exposure	Patient origin and time in non-endemic area in immigrants from Sub Saharan Africa	Comorbidities	Several risk factors investigated; age, patient origin, health care delay and others	Malaria diagnosed in Sweden
Outcomes	1.Severe *P. falc* malaria (WHO 2010) 2. Criteria for poor prognosis 3. ICU care	Severe *P. falc* malaria (WHO 2012) +/- hyper-parasitaemia 5%	1. Severe *P. falc* and non-*falciparum* malaria (WHO 2015) 2. Criteria for poor prognosis 3. Case fatality ratio	1. Lymphoid neoplasms 2. All-site non-haematological cancer

3.2 SETTING

3.2.1 Management of malaria in Sweden

Malaria is generally managed by Infectious Diseases specialists, although children mainly are treated by paediatricians and pregnant women in the last trimester at obstetric departments. The acute diagnostics is often made in the Emergency department. Other clinics may also manage malaria patients in hospitals lacking Infectious Diseases departments or when certain complications require other specialists such as kidney failure or arrhythmias. Patients with severe malaria are recommended to be managed at Intensive Care Units (ICUs). Most patients diagnosed with malaria in Sweden are admitted to hospital which is also the recommended management today, but has not always been, and asymptomatic semi-immune individuals are sometimes managed on an outpatient basis.

3.2.2 Malaria diagnostics

Diagnosis was in the majority of cases confirmed by microscopy. At most hospitals in Sweden microscopy is performed at microbiology departments during office hours, and sometimes the slides are sent to another hospital for confirmation of diagnosis. Outside office hours' diagnostics is carried out by on call Infectious Disease physicians. Of note, some hospitals in Sweden have previously not had access to a microbiology department with malaria microscopy thus depending entirely on the treating clinician to do the diagnostics, which could affect the sensitivity of the notification system.

Antigen based malaria rapid diagnostic test were often used as a complement to microscopy, especially as acute diagnostics outside office hours and in smaller hospitals without direct access to microscopy. However, microscopy always needs to be carried out for confirmation. PCR was occasionally used to determine species, especially in low parasitaemia or mixed infections where parasite determination is difficult by microscopy. Of note, *P. knowlesii*, can only be differentiated from *P. malariae* by PCR, and possibly *P. knowlesii* is underdiagnosed in our study population.

3.2.3 Study Populations

The study population of malaria patients was gradually expanded during the doctorial project (Fig 8). Starting off with adults diagnosed with *P. falciparum* in Stockholm 1995-2013 (study I), then expanding to include adults with *P. falciparum* diagnosed at the majority of Infectious Disease clinics in Sweden until April 2013, and May 2015 for Stockholm (study II), and finally all cases of malaria from all hospitals throughout Sweden 1995-2015 (III). In all studies, cases were identified through the National surveillance database at the Public Health Agency of Sweden, and additional unreported cases were identified through local microbiology and infectious disease departments. In study IV we also used the National Patient Register (NPR, see below for explanation), to identify additional cases of malaria not notified to the Public Health Agency, and included patients diagnosed 1987-2015.

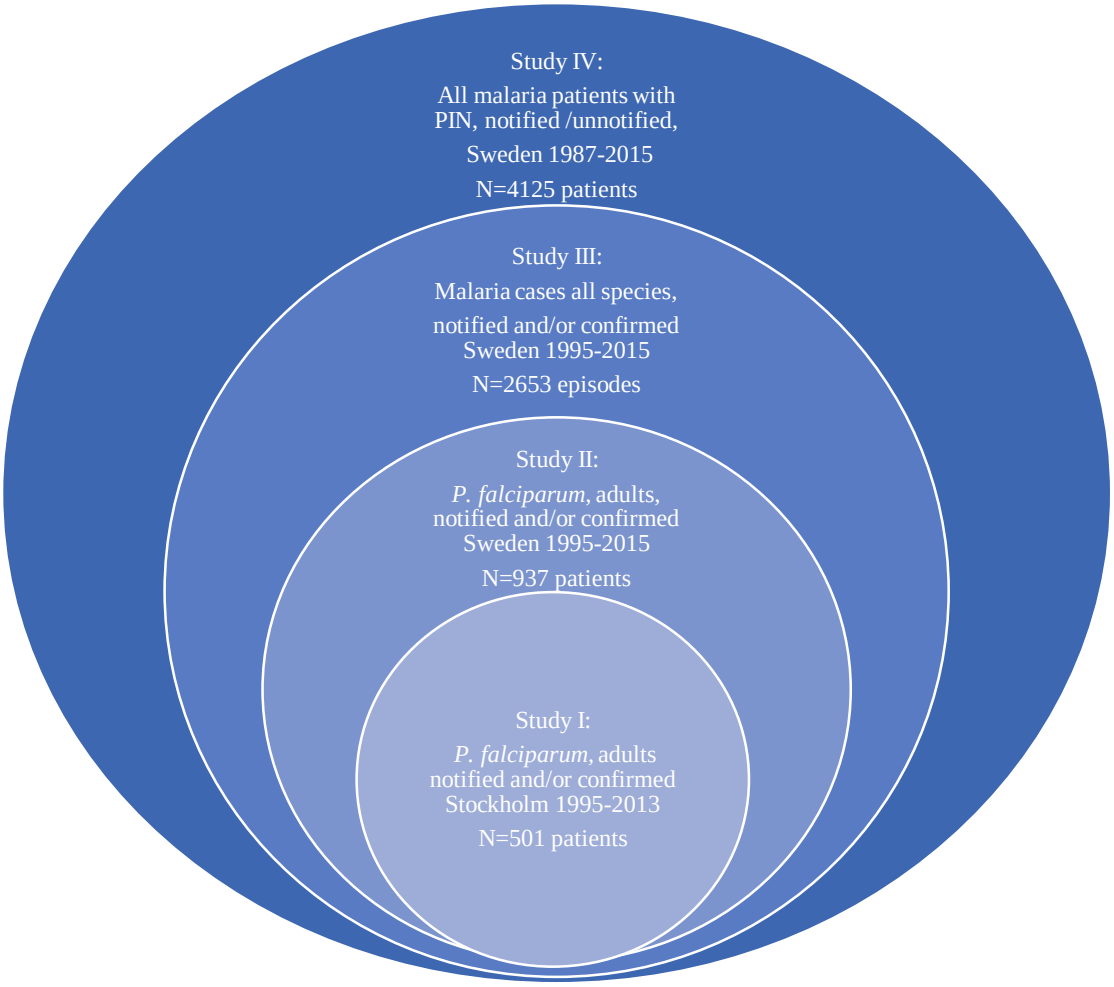

Figure 8. Schematic view over study populations in paper I-IV

3.3 DATA SOURCES

3.3.1 Patient records

We retrospectively retrieved data regarding socio-demography, travel history, chemoprophylaxis (categorized as non-use, inadequate/irregular and no use), previous comorbidities, clinical presentation including criteria for severe disease, patient and health care delay, intensive care, duration of hospital stay, treatment and outcome, from medical records in both electronical and paper form. Health care delay was defined as number of days from first health care contact until malaria diagnostics was performed, even if a first test was negative, patient delay number of days of continuous symptoms before seeking health care, based on patient report at first contact with health care.

Routine blood chemistry and microbiology data including parasitaemia, HIV and hepatitis status was also collected from both patient and laboratory records. Medication lists and previous diagnoses registered as ICD-codes in electronic patient records were reviewed to capture additional chronic diseases and optimize correct classification of comorbidity status.

Data on weight and height was recorded when present at time of malaria diagnosis for patients diagnosed in Stockholm and Umeå.

The clinical data was used in study I-III and to enrich the dataset in study IV.

3.3.2 National registers

In study IV malaria cases were linked to several national registers described below. Statistic of Sweden has demographic and socioeconomic data on all inhabitants registered in Sweden while the National Board of Health and Welfare provides statistics about diseases. All residents have a unique 10-digit identification number, the so called Personal Identification Number (PIN) assigned at birth or immigration, that is recorded throughout all contacts with healthcare providers and authorities in Sweden, enabling linkage of the malaria cases to the national registers. Patients without a PIN, such as recent arrived immigrants or temporary visitors from other countries, cannot be followed in the registers and could thus not be included in study IV.

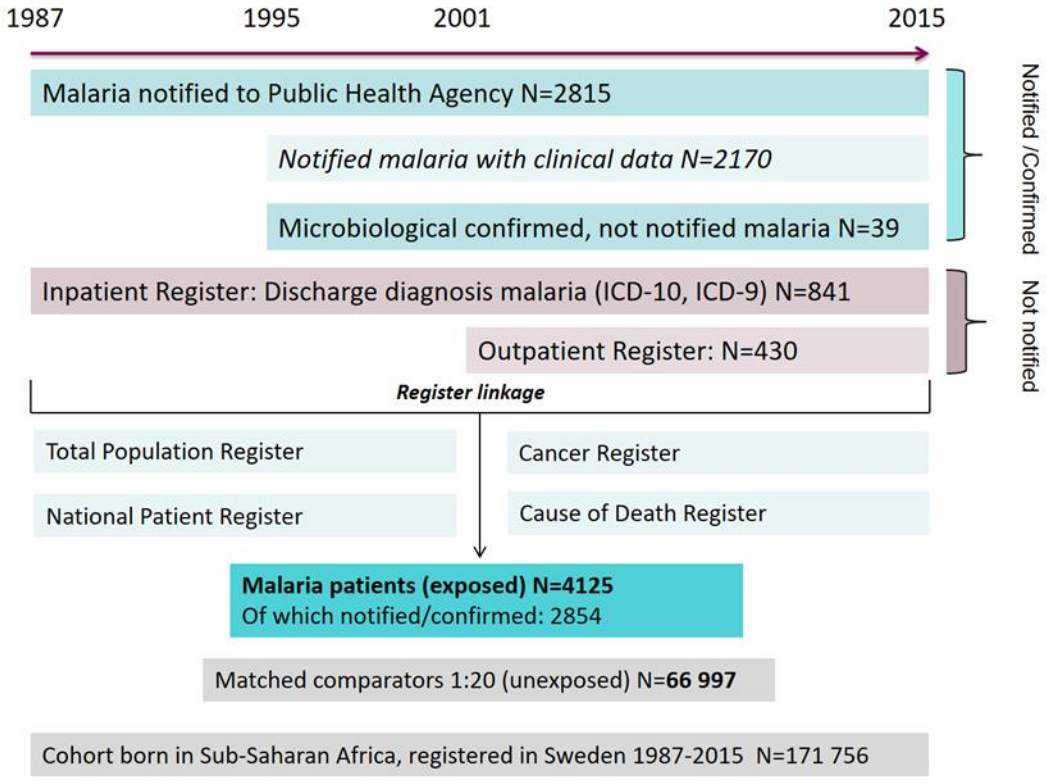

Figure 9: Overview of study cohort, data sources and register linkage process in study IV

3.3.2.1 National Patient Register (NPR)

The NPR is a population-based register at the National Board of Health and Welfare that contains information on all inpatient care since 1964, with nationwide coverage since 1987. It is estimated that 99% of all hospitalizations in Sweden are included in the National Inpatient Register. The register includes the main discharge diagnosis and up to 8 secondary diagnosis

and validation has estimated 85-95% of the diagnosis to be correct [247]. Since 2001 specialized outpatient care also is included. The NPR was both used for identification of malaria cases according to malaria-specific codes from the International Classification of Diseases 9th or 10th revision (ICD-9; 084A-H, 084W, 084X or ICD-10; B50.0-B54.9) as well as for capturing HIV and chronic hepatitis B or C diagnosed before or during follow up.

3.3.2.2 Total Population Register (TPR)

The TPR at Statistics Sweden contains demographic data for all inhabitants since 1969 including sex, date and country of birth, date of death as well as information on migration such as dates and countries of immigration and emigration, and is continuously updated.

3.3.2.3 Cause of Death Register

The Cause of Death Register at National Board of Health and Welfare contains information on date, place and both main and contributing causes of death since 1961.

3.3.2.4 Cancer Register

The Cancer Register at National Board of Health and Welfare contains information on all incident cancer diagnosed since 1958, including date of diagnosis, tumour site and histopathologic characteristics. Both clinicians and pathologists have to report a newly diagnosed cancer separately, and the estimated coverage for solid tumours is >98% [248]. The cancers are coded according to International Classification of Diseases 7th, 9th and 10th revision, but only ICD-7 codes are available for the whole period.

3.4 DEFINITIONS OF EXPOSURES AND OUTCOMES

3.4.1 Malaria endemicity

In all four studies study participants were categorized according to the endemicity of malaria in their country of birth, as a way to estimate previous malaria exposure. The UN-regions Western Eastern, Central and Southern Africa, also defined as Sub-Saharan Africa (SSA), were classified as highly endemic, based on the malaria case incidence rate estimated by WHO [249]. Low endemic areas were, due to small numbers, analysed combined with malaria free countries in most analyses.

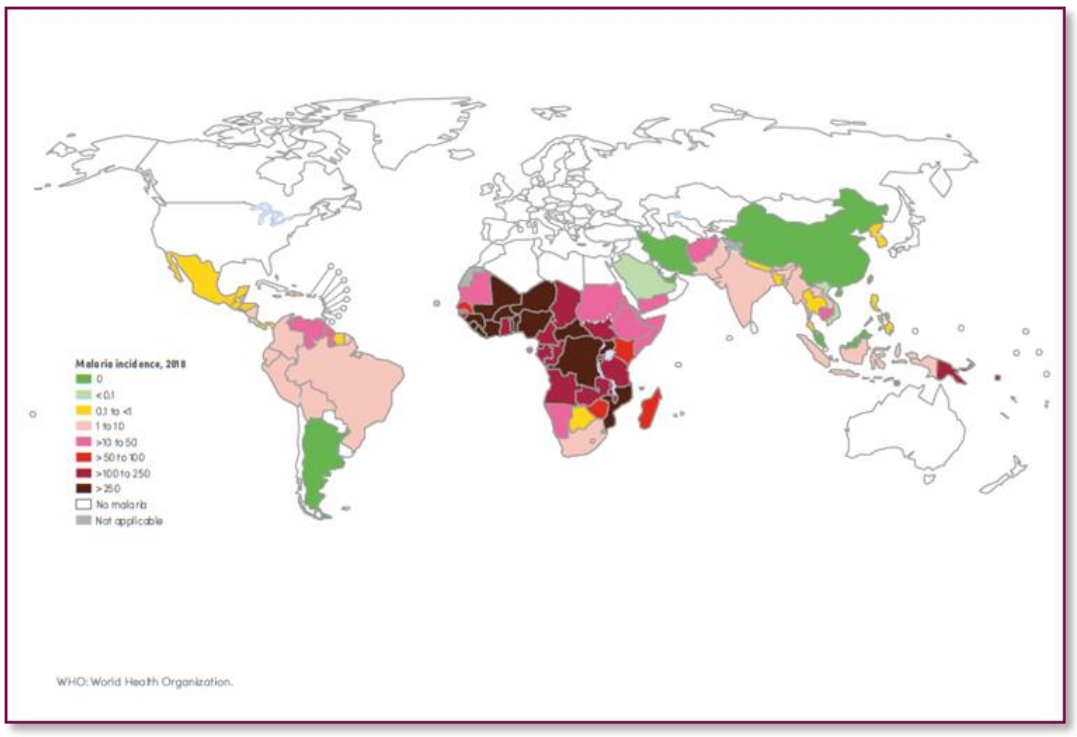

Figure 11: Malaria case incidence rate (cases per 1000 population at risk) by country, 2018
Source: World malaria report 2019. Geneva: WHO; 2019. Licence: CC BY-NC-SA 3.0 IGO.

3.4.2 Comorbidity

Medical conditions were categorized according to the International Classification of Diseases version 10 (ICD-10). Comorbidity was assessed both based on individual diagnoses and on severity weighted scores using the Charlson comorbidity index; with adjustment to ICD-10 [245]. Only chronic diseases present at the time of the malaria diagnosis and history of previous malignancies were included in the analysis. Body mass index (BMI) was calculated as weight divided by square height, and categorised according to WHO's BMI classification for adults, with obesity defined as BMI ≥30 [21].

3.4.3 Severe malaria

Since WHO has published slightly modified versions of the criteria for severe malaria, different definitions of severe malaria were also used in our studies depending on year of the study However, the differences were modest, not affecting the most severe signs, and we used the same definitions for each criteria, based on the definitions used by Bruneel et al for a large cohort of adults with imported severe *P. falciparum* treated in ICUs in multiple centres in France [77] (see Table 1 for details on criteria and modifications). In study II we used the severe criteria according to WHO 2012 [117], however since this definition did not include parasitaemia, we added a criterion of hyperparasitaemia 5% from previous guidelines, but all analyses were carried out without hyperparasitaemia as a sensitivity analysis.

Severe malaria was in all four studies defined as microbiologicaly confirmed Plasmodium species in blood and at least one of the clinical or laboratory manifestations described in Table 2.

Since the criteria are not adapted to non-endemic setting, and some of the criteria carry higher mortality risks than others [251], we also used factors with higher prognostic value for poor outcome (study I+III) as a mode to detect the most severely ill patients. These included impaired consciousness, acidosis, multiple convulsions, circulatory collapse, pulmonary oedema, and abnormal bleeding. In study III, we also included kidney failure since this criterion in later published studies has been shown to correlate to higher mortality [77, 78].

Table 2. WHO severe criteria versions 2010, 2012, 2015 [41, 117, 252] with adjustments (*).
WHO 2015 definition for severe *P. vivax* was extended to all non-*falciparum* species in study III.

WHO 2010 (Paper I)	WHO 2012 (Paper II)	WHO 2015 (Paper III-IV)
Impaired consciousness: Unrousable coma defined as GCS <11* or Blantyre coma score <3 in children		
		Prostration: Unable to sit or walk with assistance in children
Multiple convulsions: >2 episodes within 24 h		
Circulatory shock: Systolic blood pressure (SBP) <70 mm Hg in adults	SBP <80mmHg in adults	SBP <80 mmHg in adults, <70 mmHg children
Respiratory distress: Requirement of noninvasive or endotracheal mechanical ventilation or a respiratory rate of ≥40 breaths/min on room air*		Noninvasive or endotracheal mechanical ventilation, or oxygen sat <92% on room air with a respiratory rate >30 breaths/min
Acidosis: P-bicarbonate <15mmol/L, pH <7.25, base deficit >8 mEq/L or plasma lactatae ≥5 mmol/L		
Acute kidney injury: Serum creatinine >265mmol/l		
Pulmonary oedema or **ARDS** verified radiological		
Clinical jaundice or hyperbilirubinaemia: Bilirubin >50mmol/l + evidence of other vital organ dysfunction*		Bilirubin >50mmol/l +parasite count >100 000/µL
Severe anaemia: Haemoglobin concentration (hb) <50g/L	Hb <70g/L adults, <50 g/L children	Hb <70g/L adults, <50 g/L children, with a parasite count >10 000/µL
Abnormal spontaneous bleeding: Recurrent or prolonged bleeding from nose, gums, gastro-intestinal tract. Splenic rupture.		
Macroscopic heamoglobinuria		Not included
Hypoglycaemia: Blood glucose <2,2mmol/L		
Hyperparasitaemia: > 5%	Hyperparasitaemia: > 5%	Hyperparasitaemia: >10%

*WHO 2010 criteria for cerebral malaria was defined as GCS <10 but <11 was used in all studies
Respiratory distress was not defined in WHO version 2010 and 2012 why an adapted definition from Bruneel et al was used. Evidence of other vital organ dysfunction defined as circulatory instability, respiratory distress, impaired consciousness, severe coagulopathy or acute kidney injury.
In the WHO 2012 version, hyperparasitaemia was not included as a criterion for severe malaria but all analyses were done with and without >5% parasitaemia in study II

3.4.4 Lymphoid neoplasms and all-site cancers

Since ICD-7 codes are included for all cancers registered in the Cancer Register, we used ICD-7 for classification of cancers in study IV. Outcomes were defined as first diagnosis of lymphoid neoplasms (ICD-7 200-204) or first diagnosis of all-site cancer excluding heamatological malignancies and non-melanoma skin cancers (ICD-7: 140-190,192-199). Lymphoid neoplasms included Non-Hodgkins lymphoma (NHL), Hodgkins lymphoma (HL), chronic lymphatic leukemia (CLL), myeloma, acute lymphoblastic leukemia (ALL) and lymphoproliferative disorders. From 1993 histopathology diagnoses referred to as SNOMED codes (Systematized Nomenclature of Medicine) were available, and used for a more precise classification of the different types of lymphoid neoplasms as well as subclassification of

NHL, according to InterLymph consortium [253]. Histopathological codes used before 1993 are less reliable, thus subclassification of lymphoid neoplasms was only performed for those diagnosed after 1993.

3.5 STUDY DESIGN

3.5.1 Cross sectional (Study I-III)

Study I-III are all cross-sectional studies, with data collected retrospectively. Cross sectional studies use data on exposure and outcome evaluated at the same time point. Prevalence data from cross-sectional studies should generally not be used to assess causative links. However, factors that are constant (such as sex, date and country of birth and year of immigration) or known to have been there before the outcome was measured (comorbidities and BMI in study II, region of diagnosis, pregnancy and country of birth in study III), can be used as a proxy for longitudinal data, enabling more robust conclusions concerning risks or causal associations.

3.5.2 Cohort (Study IV)

Cohort studies follow a defined population (cohort) over time in order to assess if a specific exposure is associated to one or several predefined outcomes. The individuals of the cohort are classified into exposed and unexposed and all have to be free of the outcome of interest at the beginning of follow up. Each individual contributes with risk time (time from start of follow up until end of follow up) in the following analysis. Cohort studies are thus longitudinal but data can be collected both prospective or retrospective.

In study IV, malaria, defined as malaria diagnosed in Sweden, was the exposure of interest and outcomes of interest were lymphoid neoplasms and cancer. Data was collected retrospectively. The study was population based, meaning that the malaria patients were compared with individuals from the general population, and matching was done for age, gender and birth country to make the two groups as comparable as possible. Since approximately 50% of the malaria patients in Sweden are born in highly malaria endemic regions and we wanted to better control for previous exposure and pre-existing immunity, we also compiled an additional cohort composed of all individuals that were born in Sub-Saharan Africa and resident in Sweden at some point between 1987-2015.

3.6 STATISTICAL ANALYSES

Statistical analyses were done using STATA 11.0, 13.0 and 14.2. In study IV data cleaning and linkage was partly also done with SAS.

3.6.1 Descriptive analyses (study I-III)

Pearson´s chi square tests was used for comparing two or more categorical variables when there were at least 5 individuals per category; and Fisher’s exact test was used for comparing

categorical variables with sample sizes less than 5. Mann Whitney U-test was used for comparing distributions of continuous data and Kruskal-Wallis test for continuous data in more than 2 groups. A two-sided p-value of <0.05 was considered significant in all analyses.

3.6.2 Regression analyses (study I-III)

Logistic regression is used for analyses of binary outcomes. The effect measure produced odds ratio (OR), can be seen as an estimate of relative risk in case-control studies and cross-sectional studies. Logistic regression was used in study I-III to assess if one or several independent variables (patient origin, comorbidities, age, health care delay and others) were associated with severe malaria. In multivariable modelling potential confounders were included based on previous knowledge of risk factors for severe malaria (so called purposeful selection). Additional patient characteristics affecting severity in univariable analysis (with P<0.20, ref Hosmer Lemeshow) were also included (study II+III) and those variables that were not associated with severity (P>0.05) and not change the effect measure of the main predictors were removed in a stepwise backward approach. Age, number of years in endemic region and number of days of health care and patient delay were categorized. In study II (including only adults) age was instead included as a continuous variable after confirming linearity, this could not be done in study III (including children and adults) because of the u-shaped relation between age and severity. In study III patients could contribute with several episodes, thus the standard errors were estimated with the cluster-robust sandwich estimator to adjust for dependency. In study II, we tested for interaction with all variables significantly associated to severe malaria in the final model (p<0.05), and Maximum likelihood ratio test was used to determine best model fit.

Linear regression was used occasionally for assessment of continuous outcomes, such as association of patient origin with days of hospital stay in study I.

3.6.3 Cox proportional hazard regression (Study IV)

The Cox regression model uses estimated survival time of each individual to assess the probability for an outcome to occur within a narrow time span (hazard rates), thus capturing momentary risk rather than cumulative risk for the whole time period. Hazard rate ratio (HR) is the outcome measure and gives an estimation of relative risk. The advantage of the Cox model is that in contrast to most other regression models, no assumptions need to be made concerning baseline hazard function or distribution of survival time. However, the hazards compared need to be proportional over time and it is not possible to estimate the baseline hazard rate for each group.

In study IV, HR for lymphoma and cancer overall were assessed in malaria patients compared to controls from the general population. Although the two groups were matched for potential important confounders, adjustment for sex, time period of entry into study and patient origin was performed to deal with residual confounding. Attained age was used as the underlying time scale since we regarded it as the most important confounder, thus all analyses were automatically adjusted for age. Proportionality of hazard rates was tested with

Schoenefeld residuals. In the cohort with birth country in Sub Saharan Africa, the effect of malaria was analysed as a so called time-varying exposure. Accordingly, those with no known malaria diagnosis contributed with exposure free risk time only, while those diagnosed with malaria contributed with non-exposed time before date of malaria diagnosis and exposed time after that date. Sex and calendar period were included in the adjusted model. Potential interaction with total attained time in endemic region (as a proxy for previous exposure), as well as gender, was assessed in additional analysis.

3.6.4 Missing data

Most observational studies have some degree of missing data. If missing data is ignored this can lead to the introduction of bias. Data can be missed at random or systematically and traditionally missing data has been categorized into three groups based on three different mechanisms: missing completely at random (MCAR), which does not involve any bias, missing at random (MAR), which implies that the probability that a value is missing to some degree depends on other variables in a dataset, but not on the missing value itself, and not missing at random (NMAR) which implies that the probability that the value is missing depends on the missing variable value itself. In reality missing data is hardly ever completely at random and often there is some degree of NMAR in MAR. It is also difficult in practice to determine if missingness is dependent on the missing values. There are different statistical solutions for handling missing data and if there is more than 10% missing data, an imputation model is recommended [255]. In study II, where we had a large proportion of missing BMI values, multiple imputation with chained equation [256], based on variables related to severe malaria, obesity status and missingness of BMI was applied (for details concerning included variables see Suppl to paper II).

3.7 EPIDEMIOLOGICAL CONCEPTS

The main focus of epidemiological studies is to evaluate if certain groups of individuals with an exposure (which can be both external factors; such as chemoprophylaxis use, or internal; for example diabetes) are associated with predefined health related outcomes such as death, disability or a specific disease. The goal is to determine if a causal association exists –but interpretation of the results is often complicated by some fundamental epidemiological concepts that are partly explained in this section. All studies will have some degree of both systematic and random errors but with a suitable study design and adequate statistical methods these can be minimized.

3.7.1 Random errors

These are errors in measurements or variations in statistical estimates occurring by chance, that are not dependent on either exposure or outcome. If the variations of a measurement are large we attain low precision which affects internal validity of the study. By increasing sample size we can reduce random variation. We use statistical methods to estimate the

precision of our measurements based on the variations observed in the study. Confidence intervals (CI) are used to describe the range of uncertainty of an estimate; the narrower the interval the more precise the estimate. Usually, as for the studies in this thesis, the CI is set to 95%, which means we can be 95% confident that the true estimate lies within the calculated range. P-value describes the probability of attaining a result that lies within this range if there wouldn't be any difference between the groups compared (so called 0-hypothesis). A significance level may be set that specifies the degree of ascertainment we can discard the 0 hypothesis with. We used the commonly set level of 5% (0.05), meaning that if the p-value is below 0.05 we can be fairly confident that the difference observed between two groups is not because of chance, given there is no multiple testing.

3.7.2 Systematic errors - Bias

Errors that due not occur by chance but are dependent on either exposure variable or outcome are more serious than random errors since they can result in incorrect conclusions concerning the relation between exposure and outcome. Systematic errors are not affected by sample size. In epidemiology several different types of bias can occur, the most relevant to this project are briefly outlined below.

Selection bias- Implies a systematic error in the selection or inclusion of study participants. For example, if only were to include malaria patients admitted to hospitals we would have missed the less severely ill malaria patients that are treated at outpatient clinics, resulting in an overestimation of severe malaria prevalence.

Information bias –Misclassification of exposure or outcome in study participants. An example is that individuals born in malaria endemic regions likely have had malaria although not diagnosed in Sweden, thus incorrectly classified as unexposed. This may have led to a dilution of effects between those classified as malaria exposed and unexposed in the Sub-Saharan Africa cohort.

In populations with a large proportion of migrants two special concepts of bias have been described:

Healthy migrant effect –a type of selection bias implying that the immigrants that have managed to move to a new country are a healthier selection [257], and this would, at least in the initial follow-up lead to lower risk of disease and death compared to the general population.

Salmon effect – meaning that immigrants would return to their home country before death, which also would lead to underestimation of mortality and possibly other diseases. This is a form of survival bias that has however mostly been described in South American immigrants in the US and is less likely to apply for residents of African origin in Sweden that are included in the free health care system [258, 259].

3.7.3 Systematic errors -Confounding

Confounding occurs when a factor that is both associated with the exposure variable and the outcome but not on the causal pathway between exposure and outcome. Confounding is the most important concept to consider in both design, analysis and interpretation of epidemiological studies since it implies that a correlation between the exposure of interest and outcome actually is explained by another factor. Age is an important confounder in almost all epidemiological studies. If not adequately addressed, it would for example lead to overestimation of the association between comorbidities and severe malaria (study II) and most likely an underestimation in the assessment of the association between malaria and lymphoma (study IV). We handled confounding through several different methods; adjusting (study I-IV), stratification (study I-IV), matching (study IV), and by including attained age as the underlying time-scale in all analyses of study IV. In study IV, HIV and chronic hepatitis were identified as potential important confounders for lymphoma in patients with malaria. Since previous HIV and hepatitis status was not known for a large part of the cohort, but diagnosed for several individuals during the course of follow up, we chose to adjust by excluding patients with previous known HIV/hepatitis and censoring those that were diagnosed with these co-infections during follow up.

3.7.4 Effect modification

Effect modification or interaction implies that the effect of an exposure varies for different strata; for example, we suspected that the association between comorbidity and severity could be modified by previous exposure (patient origin) in study II. Interaction can be investigated through statistical methods such as stratifying for the effect modifier (I-IV) or including it as an interaction variable in a statistical model (study II+IV). In study I, we investigated, through stratification, if time in a non-endemic country is an effect-modifier for the risk of severe disease in malaria patients of endemic origin. In study IV, years in an endemic country before immigration to Sweden, was included as an interaction variable to assess if long duration, as a marker of previous probable high malaria exposure, would additionally increase the risk of lymphoma.

3.8 ETHICAL CONSIDERATIONS

The studies have been approved by the Ethical Review Board in Stockholm, Sweden (2009/1328-31/5, 2010/1080-32, 2012/1155-32, 2017/383-32 and 2017/1006-32). This allowed us to review medical records, provided by the head of respective department, without informed consent from the patients. This can be viewed as a potential threat to the personal integrity. However, review was done retrospectively following a strict protocol by a restricted number of research persons and could not affect treatment or follow-up. Since the study period spans across 20 years and the study population includes a large proportion of individuals with foreign origin and temporary stay in Sweden, as well as individuals who are no longer alive, an informed consent would have been difficult to obtain for many

individuals. This would have resulted in a large loss of data with inconclusive data analysis and interpretation.

The register linkage in study IV was performed by Statistics of Sweden and National Board of Health and Welfare and in order to assure confidentiality, the PIN was replaced by serial numbers. Hence, the data for study IV has been analyzed without PIN after linkage, and none of the study subjects would risk having their identity revealed. The Swedish national registers have been debated since data is gathered without informed consent. However, the system provides unique possibilities for systematical follow up with nationwide coverage, enabling improved management and outcome of many diseases, and would not be possible if consent from every individual would be required. Considering the substantial effects malaria has on global health, we believe that the results from these four studies will compensate for the potential infringement of personal integrity.

4 RESULTS

4.1 STUDY I.

In this retrospective case series study we reviewed 948 cases of malaria diagnosed in Stockholm 1995–2013 and identified 501 adult symptomatic patients with microbiological confirmed *P. falciparum*. 186 patients born in Sweden and other non-endemic countries were compared with 315 immigrants from Sub-Saharan Africa with different durations of residency in Sweden. The primary outcome was severe malaria according to three different definitions: WHO criteria according to 2010 definition [252], admission to the ICU, and specific factors with higher prognostic value for poor clinical outcome [251].

Among the African immigrants, 18% were newly arrived immigrants or temporary visitors with <1 year stay in Sweden, 17% had resided in Sweden for 1-4 years, 21% for 5-9 years and 39% for over 10 years. Important differences in the two groups were that the patients from Sub-Saharan Africa were younger and predominantly male, had a longer time from symptom onset to seeking healthcare and a lower usage of antimalarial chemoprophylaxis than patients born in non-endemic areas (all $p < 0.01$). Among all patients, 41 (8.2%) had severe malaria according to WHO criteria, 22 (4.4%) had factors prognostic of poor outcome, and 35 (7.0%) were admitted to ICUs.

Overall, patient origin did not affect the odds of severe malaria (OR 1.36, 95% CI 0.71–2.59), also after adjusting for sex, age, intake of chemoprophylaxis and patient delay (OR 0.89, 95% CI 0.40–1.96). And although patients of non-endemic origin tended to have higher odds for severe malaria with factors prognostic for poor outcome (OR 2.10, 95% CI 0.89–4.97) and ICU stay (OR 1.88, 95% CI 0.95–3.75), there was no association after adjustments for sex, age, intake of chemoprophylaxis and patient delay (OR 1.12, 95% CI 0.40–3.16 and 1.28, 95% CI 0.56–2.90 respectively).

However, when the African immigrants were stratified with regard to their duration of residency in Sweden, severe malaria, according to all three definitions, was less frequently observed among immigrants with <10 years stay, except for newly arrived immigrants with <1 year of stay. Time in a malaria-free country was associated with factors prognostic for poor outcome. ($p=0.02$), and immigrants from malaria endemic areas who had lived in Sweden for 15 years or more were at similar risk of the most severe manifestations of malaria as non-immune travellers, contradicting the previous hypothesis of life long immunity to severe malaria.

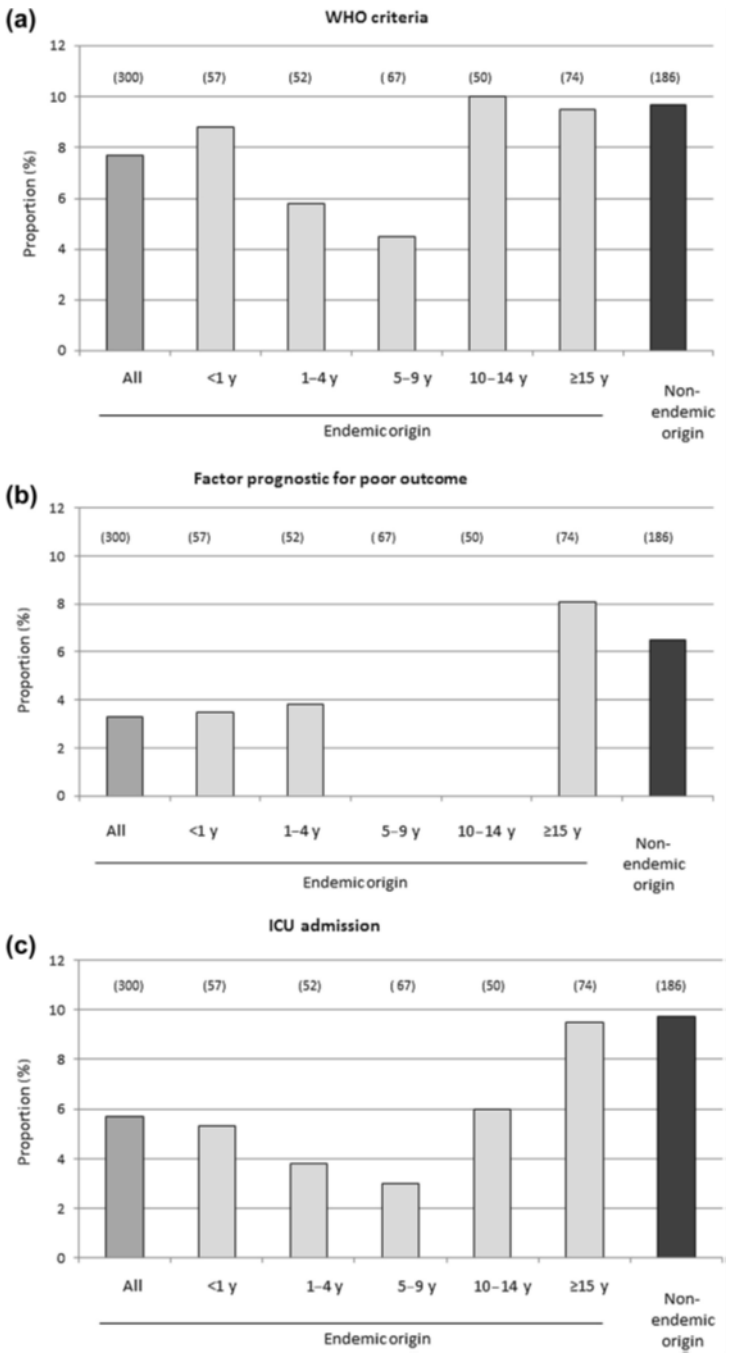

Figure 12. The proportion of immigrants with severe malaria increases with years of residency in a malaria-free country.

Severe malaria was defined according to: (a) WHO criteria, including hyperparasitaemia >5%; (b) factors prognostic for poor outcome (defined in Methods); and (c) intensive-care unit (ICU) admission.

Absolute numbers are presented above bars within brackets.

Comparison of years in a non-endemic country by the use of chi-squared or Fisher's test as appropriate, and comparison of 1–4, 5–9, 10–14 and ≥15 years, respectively, demonstrates overall differences between these four groups. p-values:(a) 0.57, (b) 0.02, and (c) 0.33.

4.2 STUDY II.

In this study we investigated if comorbidity, in terms of chronic diseases and obesity, is associated with severe *P. falciparum* malaria.

We systematically reviewed medical records from adults aged ≥18 years with microbiological confirmed *P. falciparum* diagnosed and treated at 18 hospitals in Sweden between January 1, 1995 and May 31, 2015. Comorbidities were assessed as individual diagnoses categorized according to the ICD tenth revision (ICD-10) as well as with severity-weighted scores according to the Charlson comorbidity index. Primary outcome was severe malaria defined according to WHO criteria 2012 and hyperparasitaemia >5%, and we also performed a sensitivity analysis excluding hyperparasitaemia as single criterion.

In total, 937 adults (median age 37 years; 66.5% male) were included, 92 (9.8%) with severe malaria. Patients with severe malaria had more often a chronic disease (28/92, 30.4%) compared to non-severe cases (151/845, 17.9%, p=0.004) and having 2 or more chronic diseases or a Charlson index score ≥1 was associated with severe malaria in multivariable analysis adjusted for age, healthcare delay and patient origin (adjusted OR [adjOR] 2.49, 95% CI 1.10–5.68 and adjOR 2.63, 95% CI 1.45-4.77 respectively). Among non-communicable diseases, diabetes (including type 1 and 2), cardiovascular disease and hypertension were associated to severe malaria in uni-variable analysis, however after adjustments, only diabetes was independently associated with severity (adjOR 2.98, 95% CI 1.25-7.09), with a stronger association after excluding hyperparasitaemia >5% (adjOR 3.71, 95% CI 1.49–9.20). HIV was also associated to severe malaria (adjOR 5.37, 95% CI 1.71–16.86), however not after excluding criteria of hyperparasitaemia >5% (3.42, 95% CI 0.89–13.12).

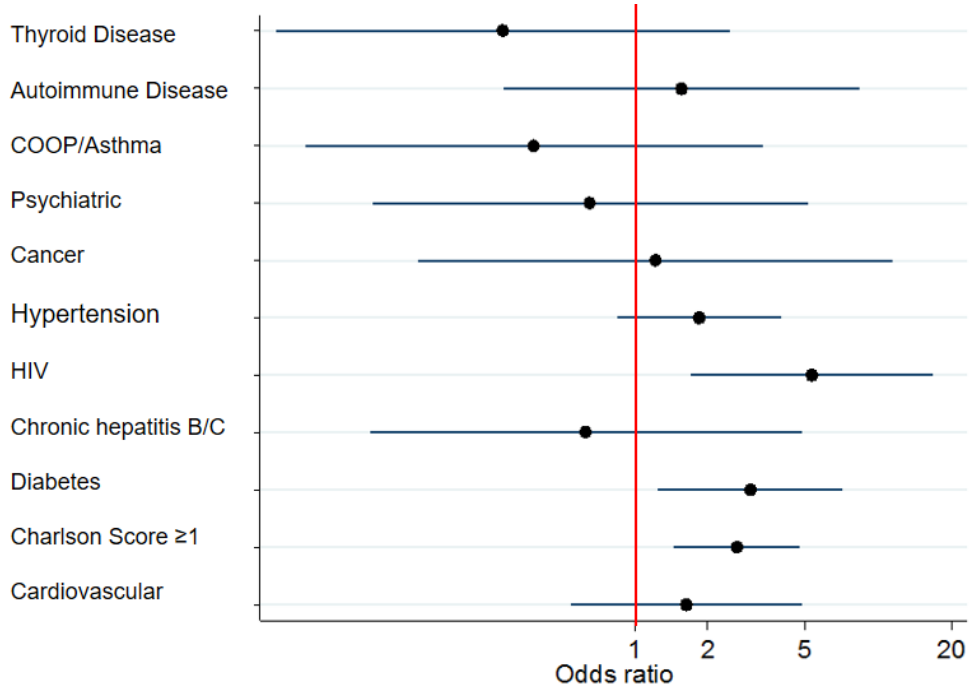

Figure 13. Comorbidities and odds ratios for severe malaria adjusted for age, patient origin and health care origin

For a subset of the population diagnosed with malaria in Stockholm and Umeå (219/569) we also had information on body mass index (BMI). Median BMI was higher among severe (29.3) than non-severe cases (24.7) (P<0.001). Obesity was strongly associated with severe malaria, both independently (adjOR 5.58, 95% CI 2.03-15.36), and in combination with an additional metabolic risk factor (hypertension, dyslipidaemia or diabetes) (adjOR 6.54, 95% CI 1.87-22.88). Excluding hyperparasitaemia >5% resulted in a stronger association between obesity and severity (adjOR 8.63 [95% CI, 2.70–27.63), as observed for diabetes.

A multiple imputation model with chained equations was performed to account for missing data on BMI, showing similar results (obesity: adjOR 4.39, 95% CI 1.66-11.58, obesity and metabolic risk factor: adjOR 5.56, 95% CI 2.53–12.21).

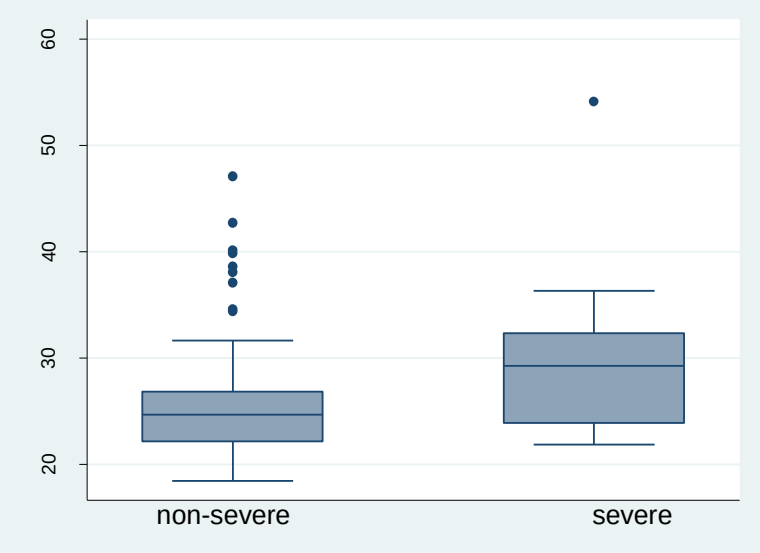

Figure 14. BMI in non-severe and severe cases of *P. falciparum* diagnosed in Stockholm and Umeå.

Both diabetes and obesity were most frequently observed among patients of endemic origin with long residency (≥15 years) in Sweden. There was a tendency of stronger associations with severe malaria for both diabetes and obesity in this group of patients, but the number of patients in each strata were small and test for interaction did not reveal any significant effect modification.

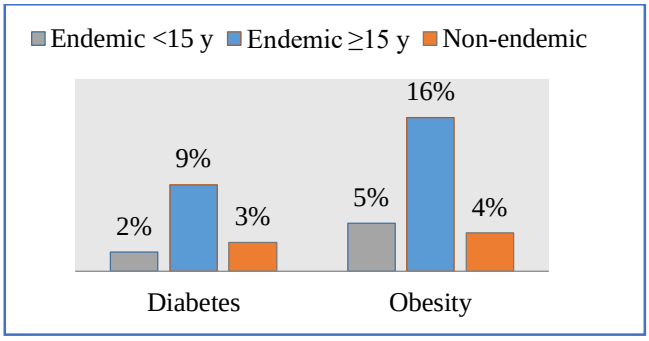

Figure 15. Proportion of patients with diabetes and obesity according to patient origin and years of residency in a malaria-free country.

4.3 STUDY III.

This is a nationwide retrospective observational study of notified and/or microbiological confirmed malaria diagnosed in Sweden 1995-2015, including all ages and species. Medical records from 2793 (85.7%) of 3260 notified and/or confirmed episodes were systematically reviewed and detailed demographic and clinical data recorded. Severe malaria was defined according to WHO criteria 2015 and similar criteria prognostic of poor outcome prognosis as used in study I [251, 77].

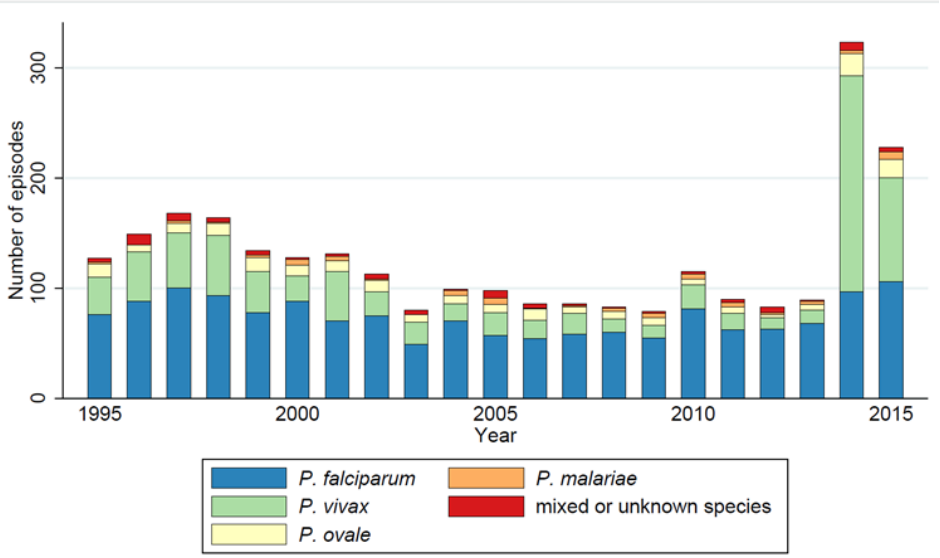

Figure 16a: Annual number of imported malaria episodes in respective *Plasmodium* species 1995-2015.

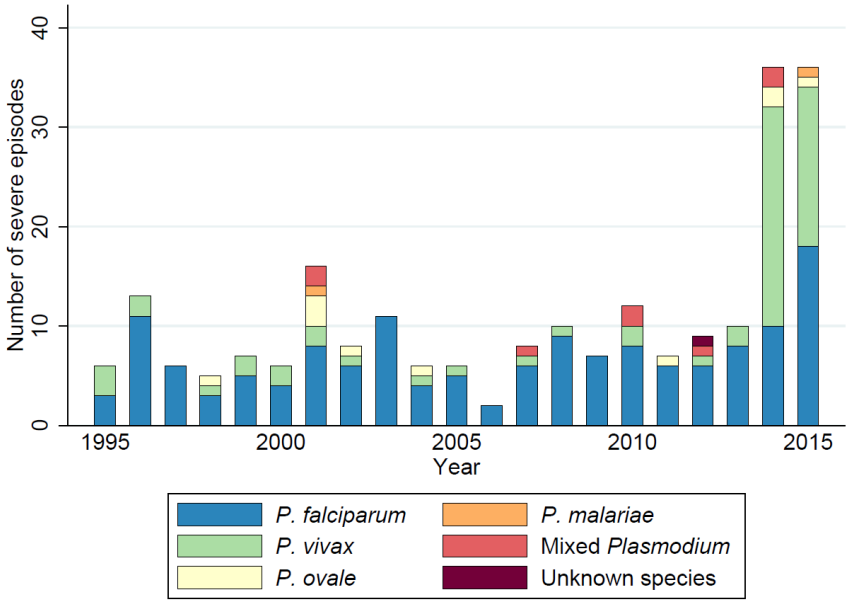

Figure 16b: Annual number of severe malaria episodes in respective *Plasmodium* species 1995-2015. *The annual number of both all malaria episodes and severe episodes increased markedly in 2014 and 2015, explained by high incidence of newly arrived Eritrean migrants with P. vivax as well as a high proportion of severe malaria in this group.*

A total of 193/2653 (7.3%) episodes fulfilled the WHO criteria for severe malaria at presentation, and an additional 34 patients deteriorated after treatment resulting in a total of 227/2653 (8.6%) severe episodes in 226 patients. These were distributed in 9.4% (146/1548) of *P. falciparum*, 7.7% (60/776) *P. vivax*, 5.3% (10/188) *P. ovale*, 3.3% (2/61) *P. malariae* and 21.1% (8/38) of mixed *P. falciparum*, and one unknown species, thus 35% of all severe episodes were non-*falciparum*. However, criteria of poor prognosis were more often found in severe *P. falciparum* compared to non- *falciparum* episodes (57.5% vs 31.9%, p<0.001) as was a combination of ≥2 WHO criteria, not including hyperparasitaemia, (40.6% vs 6.9%, p < 0.001). Overall, 127 of 2653 (4.8%) patients were admitted to ICU, but only 92 (72.4%) of these fulfilled at least one of the WHO criteria for severe malaria.

Table 3. Severe malaria criteria in episodes of different species

Severe malaria n=227[a]	***P. falciparum* n=146/1548 (9.4%)**	***P. vivax* n=60/776 (7.7%)**	***P. ovale* n=10/188 (5.3%)**	***P. malariae* n=2/61 (3.3%)**	**Mixed [b] n=8/44 (18.2%)**
WHO criteria					
Impaired consciousness	28	1	1	0	1
Prostration	9	1	0	0	0
Multiple convulsions	9	0	0	0	0
Acidosis	21	0	0	0	0
Hypoglycemia	2	0	0	0	0
Severe anemia	16[c]	15	0	1	4
Renal impairment	31	1	0	0	3
Hyperbilirubinemia	66[c]	28	6	0	4
Pulmonary edema	15	4	2	0	0
Significant bleeding	17	5	1	0	1
Shock	33	8	2	1	3
Hyperparasitemia	57	0	0	0	3
Numbers of criteria fulfilled per episode of severe malaria[d], n (%)					
1	79 (59.4)	57 (95.0)	8 (80)	2 (100)	4 (50.0)
2	25 (18.8)	3 (5.0)[e]	2 (20)[f]	0 (0)	1 (12.5)
3	15 (11.3)	0 (0)	0 (0)	0 (0)	0 (0)
4	5 (3.7)	0 (0)	0 (0)	0 (0)	2 (25.0)
≥5	9 (6.8)	0 (0)	0 (0)	0 (0)	1 (12.5)
Criteria prognostic of unfavorable outcome[g], n (% of all cases)	84 (5.4)	18 (2.3)	4 (2.1)	1 (1.6)	5 (11.4)
Fatal outcome, n (% of the severe)	3 (2.1)	0 (0)	0 (0)	0 (0)	1 (12.5)[h]

[a] 1 episode of severe malaria with unknown species fulfilled the criteria for circulatory shock

[b] Of the mixed infections including *P. falciparum*, 8/38 (21.1%) were severe, thus all severe episodes with mixed *Plasmodium* included *P. falciparum*

[c] In *P. falciparum*, severe anemia and hyperbilirubinemia includes parasitemia thresholds

[d] Hyperparasitemia is excluded in this comparison

[e] Consisting of: prostration and shock (1), bleeding and shock (1), hyperbilirubinemia and pulmonary edema (1)

[f] Consisting of: impaired consciousness and shock (1), pulmonary edema and shock (1)

[g] Including coma, multiple convulsions, acidosis, renal impairment, shock, pulmonary edema and significant bleeding

[h] *P. falciparum* and *P. ovale*

Almost half (32/72) of severe non-*falciparum* cases were newly arrived Eritrean refugees diagnosed during 2014-2015 with *P. vivax* infections, and with severe anaemia or jaundice as the most common presenting signs of severity.

Factors associated with severe *P. falciparum* malaria, were age <5 years and ≥40years, origin in non-endemic country, pregnancy, HIV, region of diagnosis (hospitals in other parts of Sweden than the two largest cities Stockholm and Gothenburg) and health care delay of 1 day or more. Age ≥40, health care delay, non-endemic origin and pregnancy were even stronger associated to the most severe forms of malaria including only criteria prognostic of unfavorable outcome. In non-*falciparum* episodes, endemic origin, health care delay of 3 days or more and a tendency of older age (≥60y), but not young age, were identified risk factors for severe disease. Recent arrived immigrants (<1 year stay in Sweden) were at increased risk of severe malaria among both *P. falciparum* and *non-falciparum* cases.

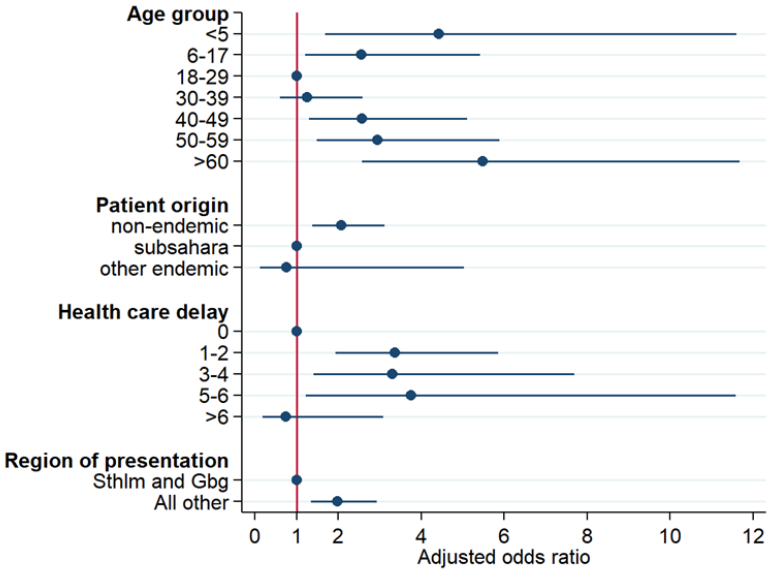

Figure 17a. Risk factors for severe *P. falciparum* malaria

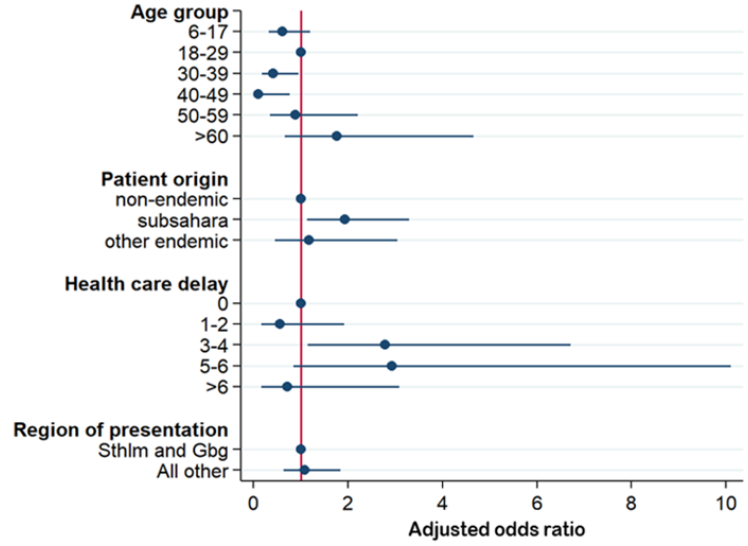

Figure 17b. Risk factors for severe non-*falciparum* malaria

Oral treatment of *P. falciparum* episodes with parasitaemia ≥2% but without severe signs at presentation were associated with progress to severe malaria with criteria prognostic of unfavourable outcome.

In total, 4 deaths occurred in the study population, corresponding to an overall case fatality rate (CFR) of 0.15% (4/2653); 0.25% (4/1582) for *P. falciparum* and 2.6% (4/154) for severe *P. falciparum*. All four patients that died were nonimmune male travelers and for two of them there was a delay in diagnosis associated to health care.

4.4 STUDY IV.

This is a population-based cohort study assessing long-term effects of malaria regarding the risk of lymphoid neoplasms and other cancers. We included all patients with complete national identification numbers diagnosed with malaria in Sweden during 1987-2015 and compared with an age, sex and birth region matched cohort without previous malaria drawn from the general population. Patients were identified through three different sources; Public Health Agency of Sweden, National Patient Register and additional cases reported by Departments of Microbiology or Infectious Diseases. An additional cohort with all individuals born in Sub-Saharan Africa and registered in the Total Population Register 1987-2015 was retrieved for separate analysis.

In total, 4 125 malaria patients and 66 997 controls were followed for an average of 13 and 14 person-years, respectively. During follow-up, 20 lymphoid neoplasms and 202 non-haematological cancers occurred among malaria patients, while 304 lymphoid neoplasms and 3933 cancers in the comparator cohort. The risk for lymphoma was not significantly increased when comparing all malaria patients with controls (HR 1.24, 95% CI 0.79-1.94). However, when stratifying the cohort according to birth region there was a tendency of higher risk for lymphoid neoplasms among malaria patients with origin in Sub-Saharan Africa (HR 1.97, 95% CI 0.81-4.81).

Table 4 Crude hazard ratios (HR) for incident lymphoid neoplasm in patients diagnosed with malaria compared to matched population controls without previous malaria diagnosis, stratified by sex, age, follow up time, years of entry and region of birth.

	Malaria, all sources[1] N=4 125			Confirmed malaria only[2] N=2 854		
	Lymphoid neoplasms among malaria patients, n (%)	Lymphoid neoplasms among comparators, n (%)	HR (95% CI)	Lymphoid neoplasms among malaria patients, n (%)	Lymphoid neoplasms among comparators, n (%)	HR (95% CI)
All	20	304	1.24 (0.79-1.94)	14	194	1.26 (0.73-2.16)
Sex						
Male	13 (65.0)	210 (69.1)	1.12 (0.64-1.95)	9 (64.2)	142 (73.2)	1.09 (0.56-2.14)
Female	7 (35.0)	94 (30.9)	1.49 (0.69-3.20)	5 (35.7)	52 (26.8)	1.68 (0.67-4.22)
Follow up time (y)						
<5	5 (25.0)	82 (27.0)	0.91 (0.37-2.24)	2 (14.3)	45 (23.2)	0.64 (0.16-2.65)
5-9	5 (25.0)	78 (25.7)	1.06 (0.43-2.62)	4 (28.6)	48 (24.7)	1.30 (0.57-3.60)
10-19	7 (35.0)	108 (35.5)	1.24 (0.58-2.67)	7 (50.0)	80 (41.2)	1.45 (0.67-3.14)
20-30	3 (15.0)	36 (11.8)	1.38 (0.42-4.51)	1 (7.1)	21 (10.8)	0.86 (0.12-6.43)
Years of entry						
1987-1994	9 (45.0)	109 (35.9)	1.51 (0.77-2.99)	6 (42.9)	67 (34.5)	1.69 (0.73-3.90)
1995-2004	6 (30.0)	155 (51.0)	0.73 (0.32-1.66)	5 (35.7)	103 (53.1)	0.81 (0.33-2.00)
2005-2015	5 (25.0)	40 (13.2)	2.29 (0.90-5.80)	3 (21.4)	24 (12.4)	1.90 (0.57-6.33)
Country of origin						
Sub Saharan Africa	6 (30.0)	25 (8.2)	1.97 (0.81-4.81)	6 (42.9)	21 (10.8)	2.32 (0.94-5.76)
Non/low endemic	14 (70.0)	279 (91.8)	1.05 (0.61-1.80)	8 (57.1)	173 (89.2)	0.91 (0.45-1.85)

[1] Confirmed and unconfirmed cases without previous lymphoid neoplasms.

[2] Confirmed malaria = notified cases from Public Health Agency Register or microbiological confirmed

There was no increased risk, but rather a tendency of decreased risk, for non-haematological cancers in malaria patients (HR 0.89, 95% CI 0.77-1.02). When investigating the distribution of cancer types according to malaria exposure and region of birth, the proportion of lymphoid neoplasms was found to be considerable increased among malaria patients with Sub Saharan origin (18%) compared to both comparators with same origin (9%), as well as malaria patients (5%) and population comparators (6%) with non/low-endemic origin. Except a high proportion of endocrinal tumours and liver cancers among both malaria patients and comparators from Sub-Saharan Africa, no other major differences in the distribution of cancer types were observed. (Table 5)

Table 5 Types of cancers in patients with malaria and comparators without previous malaria diagnose, according to birth origin.

Type of cancer	Malaria patients, non-endemic origin n (%)	Matched comparators, non-endemic origin n (%)	Malaria patients, endemic origin n (%)	Matched comparators, endemic origin n (%)
All	190	3976	33	237
oropharynx	3 (1.6)	75 (1.9)	0	5 (2.1)
stomach	1 (0.5)	62 (1.6)	1 (3.0)	5 (2.1)
liver	2 (1.1)	63 (1.6)	1 (3.0)	12 (5.1)
colorectal	19 (10.0)	407 (10.2)	3 (9.1)	17 (7.2)
Gastrointestinal +other	5 (2.5)	136 (3.4)	1 (2.7)	8 (3.3)
respiratory	9 (4.7)	259 (6.5)	2 (6.1)	17 (7.2)
breast	19 (10.0)	380 (9.6)	3 (9.1)	17 (7.2)
female genitals	30 (15.8)	605 (15.2)	6 (18.2)	28 (11.8)
male genitals	50 (26.3)	868 (21.8)	5 (15.1)	37 (15.6)
urinary tract	8 (4.2)	234 (5.9)	0	11 (4.6)
melanoma	17 (9.0)	289 (7.3)	1 (3.0)	3 (1.3)
CNS +eye	1 (0.5)	121 (3.0)	0 (0)	10 (4.2)
endocrinal	6 (3.2)	84 (2.1)	2 (6.1)	30 (12.7)
Bone +soft tissue	0	36 (0.9)	1 (3.0)	4 (1.7)
lymphoma	9 (4.7)	218 (5.5)	6 (18.2)	22 (9.3)
leukemia	5 (2.6)	44 (1.1)	0	5 (2.1)
myelofibrosis	0	21 (0.5)	1 (3.0)	3 (1.3)
unknown primary	6 (3.2)	75 (1.9)	0	4 (1.7)

When assessing all individuals born in Sub-Saharan Africa resident in Sweden, we observed an increased risk for lymphoid neoplasms after malaria diagnosis (HR 2.39, 95% CI 1.06-5.40), also after adjustment for sex and calendar period (adjHR 2.33, 95% CI 1.03-5.26), but no difference in the risk of non-haematological cancers (HR 1.01, 95% CI 0.70-1.45, adj HR 1.14, 95% CI 0.79-1.63). The increased risk of lymphoid neoplasms could not be explained by co-infection with HIV or hepatitis B or C, since the risk estimate was similar after excluding these patients (HR 2.63, 95% CI 1.08-6.42, adjHR 2.50, 95% CI 1.02-6.11). The association became more pronounced when restricting the analysis to confirmed cases only (HR 2.97, 95% CI 1.32-6.70, adjHR 2.88, 95% CI 1.27-6.51) and using a stricter definition including only Non-Hodgkin and Hodgkin lymphoma (HR 4.35, 95% CI 1.77-10.67, adjHR 4.05, 95% CI 1.65-9.96). The malaria patients with endemic origin that developed lymphoma had all notified malaria, immigrated to Sweden at 18-34 years of age and were diagnosed with lymphoma after 10 years in Sweden.

Table 6. Crude and adjusted hazard ratios for incident lymphoid neoplasm and all site non-haematological cancers in population born in Sub-Saharan Africa (SSA) with and without malaria diagnosis. All periods of registered stay in Sweden included from 1987.

Population born in SSA. Malaria all sources.[1] Periods after malaria diagnosis N=1426 Periods without malaria diagnosis N=177 721				**Population born in SSA. Malaria confirmed cases.[2] Periods after malaria diagnosis N=1165** Periods without malaria diagnosis N=177 721		
Lymphoid neoplasms in periods after malaria diagnosis, n	**Lymphoid neoplasms in periods without malaria diagnosis, n**	HR (95% CI)	adjHR[3] (95% CI)	**Lymphoid neoplasms in periods after malaria diagnosis, n**	HR (95% CI)	adjHR[3] (95% CI)
6	206	2.39 (1.06-5.40)	2.33 (1.03-5.26)	6	2.97 (1.32-6.70)	2.88 (1.27-6.51)
Population born in SSA. Malaria all sources.[4] Periods after malaria diagnosis N=1419 Periods without malaria diagnosis N=177 611				**Population born in SSA. Malaria confirmed cases.[5] Periods after malaria diagnosis N=1160** Periods without malaria diagnosis N=177 611		
All-sites cancer[6] in periods after malaria diagnosis, n	**All site cancers among unexposed, n**	HR (95% CI)	adjHR[3] (95% CI)	**All-sites cancer[6] in periods after malaria diagnosis, n**	HR (95% CI)	adjHR[3] (95% CI)
30	2376	1.01 (0.70-1.45)	1.14 (0.79-1.63)	22	0.92 (0.60-1.40)	1.04 (0.68-1.59)

[1] Confirmed and unconfirmed cases without previous lymphoid neoplasms.
[2] Confirmed malaria = notified cases from Public Health Agency Register or microbiological confirmed
[3] Adjusted for sex and calendar period. Age as underlying time-scale in all analyses.
[4] Confirmed and unconfirmed cases without previous cancer.
[5] Confirmed cases without previous cancer.
[6] Haematological cancers and non-melanoma skin cancers excluded.

5 DISCUSSION

5.1 MAIN FINDINGS

With the four studies of this thesis, the epidemiology, clinical presentation and outcome of *P. falciparum* and non-*falciparum* malaria have for the first time been systematically investigated in Sweden. More specifically, we demonstrated that severe malaria needs to be considered also in imported non-*falciparum* malaria and that newly arrived migrants is a risk group for both severe *falciparum* and non-*falciparum* malaria (**Study III**). Comorbidity, specifically obesity, diabetes and combinations of the metabolic syndrome (**Study II**), were risk factors for severe malaria in both non-immune travellers and immigrants of endemic origin diagnosed with *P falciparum.* Moreover, time of residency in Sweden affected the risk of severe malaria in immigrants from Sub Saharan Africa, adding to the understanding of the longevity of clinical immunity to severe malaria (**Study I**). Finally, we have found an increased risk of lymphoid neoplasms, in individuals from endemic areas diagnosed with imported malaria (**Study IV**).

Together, the studies in this thesis have described the presentations and risk factors for severe imported malaria in different species as well as provided evidence for long-term consequences after malaria.

5.2 ASSESSMENT OF SEVERITY –ARE ALL PATIENTS WITH SEVERE MALARIA SEVERLY ILL?

5.2.1 The severe criteria

Severe malaria was defined by WHO over 30 years ago, with criteria adapted for endemic settings, and since then there have been several modifications, and different versions have been used in different studies, complicating the comparison of risk factors for severe disease. In addition, the criteria are certainly not independent of each other and different criteria have different prognostic values, with coma, circulatory shock, and metabolic acidosis (clinically manifested as respiratory distress), having been proposed to be most relevant for outcome in endemic and non-endemic settings [69, 77, 118, 260]. More recently, acute renal failure has also been associated with increased mortality [78, 81]. In contrary, heamaturia and hyperbilirubinemia have not been shown to have any significant impact on outcome [81, 186, 260]. Correspondingly, hyperbilirubinaemia, which was the most common sign in both severe *P. falciparum* and non-*falciparum* episodes of study III, was seldom observed in association with other severe signs.

In an attempt to evaluate which patients were most severely ill, we used adapted criteria prognostic for poor outcome in addition to the WHO definition in both study I and III. In study II we instead used criteria without hyperparasitaemia as a sensitivity analysis. Among all severe *P. falciparum* episodes in study III, 58% of those defined by the WHO 2015 criteria also fulfilled criteria prognostic of unfavourable outcome. These criteria correlated better to ICU admission than the WHO criteria. In addition, criteria prognostic for poor

outcome showed stronger associations to several of the identified risk factors for severe *P. falciparum* in study III, and in study I, they best predicted an increased risk of severity among the African immigrants with long duration of residency in Sweden. Thus, we consider criteria prognostic for poor outcome to be a more robust marker of severity than the WHO criteria in our setting. We would have preferred to also assess individual risk factors in relation to specific criteria, however this would have required even larger study populations. The proportion of individual criteria for different risk groups (diabetes and obesity in Study II) and for different parasite species (Study III) will however be discussed later.

We also assessed disease severity by number of WHO criteria. In study III, only 54 of 133 severe *P. falciparum* episodes (excluding hyperparasitaemia) fulfilled at least two criteria, and a higher proportion of these were admitted to ICU (83%); compared both to those with one criterion (30%) and those with factors prognostic of unfavourable outcome (76%). Possibly, using two criteria is an even better strategy to identify those that are at highest risk of poor clinical outcome, and other studies have used a similar approach [84].

Admission to ICU is certainly also a marker of severe disease and was included in assessment of severity in study I and III. Only a third (92/227) of all severe episodes were treated at ICU, on the other hand, a third (34/129) of episodes treated in ICU were not classified as severe according to WHO criteria. Certainly, admission to ICU is affected by local guidelines in different hospitals, available beds, time period and personal judgement of individual intensivists. At the same time, Sweden is well-known for an overall low number of ICU beds per capita [261] with tough decisions usually preceding admission, indicating that at least some of these patients must have other signs of severity that are not included in the WHO definition. In addition, 34 of patients with severe malaria according to WHO definition did not present with the severe criteria at admission, but deteriorated severe malaria after initiation of treatment.

Evidently there is still a need for additional adaption of the severe criteria to better identify patients that are the most severely ill but also those at risk of deterioration, and with the greatest benefit of a higher level of monitoring and care.

5.2.2 Parasitaemia

The role of parasitaemia for disease severity and the appropriate cut-off for defining severe malaria has been much debated, partly because hyperparasitaemia has different prognostic values depending on age-group and previous immunity [262]. The WHO criteria contain varying parasitaemia thresholds in different editions of the guidelines and for different populations [41, 117, 252]. Other studies of imported malaria have shown associations with severity at 2% [188] and with fatal outcome at 4% parasitaemia [263], and Bruneel et al reported 1.4-fold increased risk of death for each 5% increase in parasitaemia [77]. However, it has also been shown that patients with fatal outcome can present with low parasitaemia [264]. Among the few cases of death in our study, parasitaemia at admission varied from 0.13%-22%, all in non-immune patients.

Among the 1548 patients diagnosed with *P. falciparum* in Sweden 1995-2015 (study III), 82% patients with hyperparasitaemia >10% also had other signs of severe malaria while only 45% of those with >5% met other criteria for severity. However, in study III we observed that patients with *P. falciparum* presenting with parasitaemia >2% and receiving oral antimalarial treatment had an increased risk of progressing to malaria with the most severe signs. In the latest version of WHO criteria [41], a hyperparasitaemia of 10% has been included as criterion irrespective of setting, thus by strictly following the guidelines also non-immune patients with up to 10% parasitized erythrocytes should be treated with oral ACT. Based on the findings of study III we recommend clinicians to consider treating patients with >2% parasitaemia with iv artesunate irrespectively of additional severe criteria, which also is in line with other European guidelines [265].

The inconsistent association between parasitaemia and severity as well as fatality in different studies, is possibly due to the sequestrating ability of *P. falciparum*, implying that the parasites counted in the circulating blood is not a fully adequate estimation of overall parasite biomass. In the future, we will hopefully be able to use more precise biomarkers for estimating parasite load, such as *P. falciparum* histidine-rich protein 2 (HRP2) in plasma [266].

5.2.3 Fatality

Death is by all means the most severe outcome. Only 4 patients died among the 2545 patients diagnosed with malaria in Sweden 1995-2015 (study III). All were infected with *P. falciparum*, one mixed with *P. ovale*, conferring a case fatality rate (CFR) of 0.15% overall and 0.25% for *P. falciparum*, which is low compared to other studies of imported *P. falciparum* malaria with CFR varying from 0.3–4% [82, 145, 147, 148, 266], although overall lower rates are seen in patients with endemic origin [27]. The fatality for severe *P. falciparum* was also low in our study (CFR 2.6%) compared to other reports of imported malaria in Europe, however there is a striking variation both within and in between countries (3.8%-15%) [77, 84, 148, 266]. In a recent European multi-centre study, CFR was estimated to only 1.6% in severe *P. falciparum*, however case inclusion was based on voluntary report to the TropNet, mostly by tertiary care hospitals with great expertise in management of severe malaria, thus this is probably an underestimation of true fatality rate in Europe [191].

With only four deceased it would not have been meaningful to use fatality in the assessment of severe risk factors, but an important observation is that all cases were non-immune male travellers, corresponding to a CFR of 7% in this particular group of severe *P. falciparum*. In addition, all were diagnosed in regions where malaria is less often managed, health care delay was involved in two of the cases and patient delay in all (2-5 days), thus there were several risk factors that have been associated with mortality in other studies of imported malaria [82, 145, 146]. As in other studies of imported malaria [267], treatment was certainly also an important factor for the fatal outcome; only one patient was initially treated with iv artesunate, two were treated with iv quinine and one with oral quinine.

Importantly, the case fatality rate in our study is based on notified cases of malaria and some deaths due to malaria might certainly have been missed. In a study reviewing malaria related deaths among US travellers, 18% of the lethal cases were diagnosed at autopsy (Newman 2004) and in the linkage to the National Cause of Death Register in study IV we identified an additional 15 individuals during 1995-2015 with malaria reported as underlying cause of death. Thus, the mortality due to imported malaria in Sweden remains to be investigated, and a population-based cohort study using information from our national registers to assess both acute and long-term mortality is being planned.

5.3 RISK FACTORS FOR SEVERITY

5.3.1 Old age

Study I-III all demonstrated a clear association between increasing age and severe *P. falciparum* malaria, starting at 40 years of age and with an adjusted OR of 5.5 for individuals aged 60 years and over (study III). This is in line with previous studies of imported *P. falciparum* malaria, reporting adjusted OR of 2.7-5.7 for age >60 [148, 183, 188], with even higher odds of up to 10.7 for fatal malaria [145], and several studies also demonstrating a step wise increased risk for each decade above 40 years [183, 188]. The highest risk for severe malaria among elders was reported in a study restricted to non-immune European travellers [183], and probably the effect of old age is more important in previous unexposed populations [268]. Although we did not report effect of age stratified by origin, migrants from Sub Saharan Africa with severe malaria were generally younger than the travellers of non-endemic origin (study I-III), and the effect of age was somewhat decreased after adjustment for patient origin (study III).

The effect of age on severity in non-falciparum cases has not been as well studied since most publications assessing severity of imported malaria only include *P. falciparum* malaria. A study of hospitalized malaria cases in the USA, analyzing all species jointly, reported that age >65 years was associated with a 4.6 times increased odds for severe malaria overall [203]. However, 83% of the severe cases with known species information were *P. falciparum.* In study III, where we assessed species-specific risk factors, age also showed a tendency to affect severity of non-*falciparum* malaria, however only in patients >60 years of age and less pronounced than for *P. falciparum.*

In addition to age-associated changes in the immune system, part of the age effect could possibly be related to specific pathophysiological effects of *P. falciparum* in different organs. Age-specific manifestations have recently been investigated in non-endemic settings, demonstrating increased risk of acute renal failure and cerebral malaria among older age-groups [191, 203]. In a large study of over 1000 severe cases in a low-endemic setting, age >50 years was associated with renal failure, hyperparasitaemia and jaundice, but not cerebral malaria [186]. Although we did not investigate if age was related to specific severe criteria, we demonstrated that increasing age was also associated with the most severe criteria prognostic of unfavourable outcome.

Finally, underlying comorbidities have been proposed as a contributing factor for the increased risk in older age groups [145], but had not been previously investigated. In study II, we demonstrated the impact of several chronic diseases for severity of *P. falciparum*, however the age effect was still observed after adjustment for these comorbidities.

5.3.2 Young age

Children living in endemic areas are at risk of severe malaria before gradually developing immunity after repeated exposure, but might constitute a risk group for severe malaria also in non-endemic settings, as indicated by high prevalence of severe cases among children with imported malaria (18-19%) [269-272]. However, few studies assessing risk factors for severe imported malaria include both children and adults. In study III, we observed that 20% (10/49) of children in age-group 0-5 presented with severe *P. falciparum* malaria and 10% (17/171) of children 6-17 years, however much lower prevalence among non-*falciparum*, with no severe cases in the youngest age group. When including children in the assessment of risk factors we observed a significantly increased risk of severe *P. falciparum* malaria in the youngest age group (0-5 years), with an adjusted OR of 4.4, thus almost as high as for the oldest age group. On the contrary, in a large study assessing risk factors for severe *P. falciparum* in France, children were not at increased risk for severe malaria, however the age group <5 years was not specifically investigated [82]. And in another study from France restricted to children only, an age of under 5 years was associated with severe malaria, but not after adjustment for other factors [272].

Notable, a significantly higher proportion of children ≤5 years were born in Sweden compared to other age groups, the majority however with parents born in an endemic country, thus previous exposure could have occurred. In addition, health care delay was also more commonly observed in children <5 years, and strongly associated with severity, as has been reported elsewhere [271-273]. This probably reflects the high proportion of nonspecific signs and symptoms of malaria presentation in children [270], together with fever being a very common cause of health care contact especially in small children.

Globally, children under 5 years are at highest risk of not only severe malaria but also death [1]. In contrast, we recorded no deaths among children with severe malaria, and in line with previous studies of risk factors for mortality in imported malaria, deaths in children is highly uncommon in non-endemic settings (reviewed in [273], most publications reporting no fatal cases among the youngest children [60, 145, 274], indicating that the reason for the high mortality seen especially in Sub-Saharan Africa, most likely is related to poor access and standard of health care. Another explanation to the discrepancy in severity and mortality in our setting could be that children with severe malaria present with less severe signs, as shown in one report, where the majority of children with severe malaria had hyperparasitaemia as only criteria [270]). Although this was not the case in study III, age ≤5 was not significantly associated to the criteria prognostic for poor outcome.

Nonetheless, the high proportion of severe cases among children aged 0-5 years, and the association with health care delay indicate that the management of children with malaria can be improved also in our setting.

5.3.3 Treatment delay

Delay in initiation of correct treatment, both due to patient delay and health care delay, has in repeated studies of imported malaria been associated to mortality, in particularly for *P. falciparum* [84, 145, 179, 181]. A few studies have also assessed treatment delay for severe malaria, with one study of imported *P. falciparum* reporting 4-12 days of delay in diagnosis being associated with severity, however no differentiation was made between health care and patient delay [148]. In study II and III, health care delay was observed in a third of severe cases, and we clearly showed that health care delay, but not patient delay, was associated to severe P. *falciparum*, with increasing risk for increasing number of days, also after adjustments for other risk factors such as age, patient origin, and comorbidity (in study II). Furthermore, we demonstrated that health care delay was also associated with criteria prognostic of unfavourable outcome and ICU admission. For non-*falciparum*, we also observed increased odds for severe malaria, however from 3 days of delay, possibly related to different pathogenesis of these species.

In addition to being more common in children, we observed a higher proportion of health care delay in females and patients of non-endemic origin (study III). For some cases of delay, we found a previous health care contact outside the hospital, most often with a general practitioner, which also has been identified as a risk factor for severe malaria in an earlier study [148]. One of the challenges with malaria in a non-endemic setting is suspecting the diagnosis, as there are no typical presentations or laboratory parameters for malaria. Evidently there is a need for raised awareness among clinicians of all specialities to ask for travel history in patients seeking with fever, irrespective of age, gender or country of birth.

Interestingly, we could not show that patient delay was significantly associated with severity neither in *P. falciparum* nor non-*falciparum* malaria (study III). A similar observation was made in an overview of imported *P. falciparum* to Norway where doctors delay, but not patient delay, was associated with complicated malaria [275]. The two largest analyses of malaria related deaths in travellers have shown a high proportion of patient delay among fatal cases [145, 181], however both of these studies were based on surveillance data with a substantial proportion of incomplete data concerning delay, especially in non-fatal cases, which could bias the results. Another contradicting finding compared with the above study by Checkley et al, was that patient delay was longer in immigrants from Africa compared to Swedish travellers (study I). Possibly, patient delay in our study population was more likely related to mild symptoms, while cases of patient delay in severely ill, associated to fear of seeking health care or other sociocultural reasons, could have been missed as they might have resulted in deaths outside the hospital, as was observed for one fourth of the fatal cases in Checkley et al [145].

Other studies have reported that patients presenting at health care facilities lacking expertise in tropical medicine, did not receive optimal treatment [184], and this has also been associated with an increased risk of fatal outcome [145]. In accordance, study III demonstrated a higher risk of severe *P falciparum* malaria for patients that presented outside the two largest regions in Sweden (where also most malaria patients are diagnosed). Of importance, this association was also seen after adjustment for health care delay, and most of the patients presented with severe criteria already at admission, thus possibly there may be differences in the management of malaria that could not be detected by the variables we have included in our assessment.

5.3.4 Pregnancy

Pregnancy is a well-known risk factor for severe malaria in women in low-endemic areas, with increased case fatality rates observed especially after the first trimester [160]. For non-endemic settings, assessment of risk in pregnancy have been scarcely reported, probably since many observational studies are based on surveillance data and lack info on pregnancy status. However, several case series [77, 163, 164], give support to that pregnancy is a risk factor for severity in non-endemic settings despite available high standard of care for both mothers and neonates.

A recent multicentre review of imported malaria in pregnant women reported only 46 cases of severe malaria in a total of 631 pregnant women (pooled estimates, all species) over a long time frame, but data on clinical presentation and outcome was lacking for over half of the women, thus the prevalence of severe malaria among pregnant women with data on outcome was 16% [165], which is comparable to the proportion observed in our population (21% in Study III). The most common complication among pregnant in this review was severe anaemia, in contrast to our study where only one woman presented with severe anaemia as only criteria, the remaining all had at least one criteria for poor prognosis. In study III, we could demonstrate that pregnancy was associated with severe malaria, however, there was no significant association in study II, probably because of a smaller study population. Nonetheless, pregnancy could not be included in the multivariate model in study III because it caused instability of the regression model and drift in the odds ratios. Study III included 13 pregnant women with non-*falciparum* malaria, however none of these had severe malaria. An interesting observation is that all pregnant women with severe malaria were of endemic origin and half were newly arrived to Sweden. In the multicentre study of pooled pregnant cases, there was no data on birth country in severe cases, however VFR was the main reason for travel (58%) among all cases, and the second most common group were newly arrived immigrants or refugees.

The observations from study III suggests that preventive measures might be indicated among pregnant women of endemic origin, such as improved travel advice for those visiting friends and relatives and possibly screening of newly arrived women from regions with high transmission. In addition, close monitoring should be considered in all pregnant women

admitted with *P. falciparum* malaria, irrespectively of origin, parasite level or signs of severity.

5.3.5 Chemoprophylaxis usage

In line with other studies [147, 268], chemoprophylaxis usage was significantly lower in travellers of endemic origin visiting friends and relatives (VFR) compared to travellers of non-endemic origin (study I-III). We did not find any association between inadequate chemoprophylaxis usage and severe *P. falciparum* malaria in study II, and only a slightly increased risk after adjustment for age, health care delay and patient origin in paper III. This is in contrast to several other studies reporting clear correlation between non-use and severe [148] as well as fatal malaria, especially in non-immune individuals [77, 145, 179, 181] However, most of these studies have a large proportion of missing data regarding chemoprophylaxis use, and together with the potential of reporting bias, the reliability of these associations has to be regarded with caution. In study III, we only had 3% missing data for this variable, possibly explaining the less pronounced effect, but the assessment of usage is still susceptible to reporting bias. In a prospective study of imported severe malaria, chemoprophylactic use was assessed by documentation of prescribed antimalarial, and they observed no significant difference between severe and non-severe cases.

There is evidence of delayed symptom onset in patients taking chemoprophylaxis, especially for *P vivax* [105, 199], however there are no data on the potential effect on severity. In study III we observed that lack of chemoprophylaxis was associated with slightly increased odds for severe malaria in non-*falciparum* infections, but possibly this association could have been confounded by the high proportion of newly arrived immigrants without chemoprophylaxis presenting with severe malaria (study III).

5.3.6 Sex

As in all studies of imported malaria [83, 147, 201], a larger proportion of men presented with malaria in study I-IV, irrespective of species (III). Female sex has been shown to have a protective effect on disease course in several infectious diseases [276] and in two large studies of imported *P. falciparum* malaria a higher proportion of males was seen among fatal cases, however no association was seen after adjustment [77, 277]. In none of our studies we could demonstrate any increased risk for severity attributed to sex, neither before nor after adjustment. Considering that health care delay occurred more often in women and pregnancy is an obvious risk factor, one would suspect that the risk for severe malaria might actually be lower than observed for non-pregnant women. To truly dissect this matter, one would need to assess severe malaria in groups stratified by age, patient origin, health care delay and chemoprophylaxis use, which would require a larger patient material.

5.4 PATIENT ORIGIN AND SEVERITY

5.4.1 Estimating previous exposure

Numerous studies have shown that travellers with origin in a non-endemic country are at greater risk of both severe and fatal malaria compared to immigrants from malaria endemic countries [82, 145, 147-149, 184, 194]. Country of origin has in these, as well as in the studies of this thesis been used as a crude marker for previous exposure and presumed immunity, with travellers born in a malaria-free countries assumed to be non-immune and African immigrants to be semi-immune. None of these previous publications have however specifically investigated if time of residency in a malaria-free country affects the risk of severe disease among immigrants with endemic origin.

In line with previous studies, we observed increased odds for severe malaria in travellers from Sweden compared to immigrants born in Sub-Saharan Africa, when an un-stratified approach was used in both study II and III. In study I we could not demonstrate any significant difference in the overall comparison, although there was a tendency of higher risk in the Swedish travellers when factors prognostic for poor outcome were used as outcome. This difference can probably be explained by the smaller study population, as well as single center vs multi-center design with different patient constitutions; 39% of the African immigrants had >10 years of residency in Sweden compared to 19% in study III. The diverging results emphasizes how important it is to consider stratifying a heterogeneous risk group to unmask differences that might be important for the outcome. In accordance, when immigrants in the nationwide cohort of study III were stratified according to time in a non-endemic area, we saw the same pattern as in paper I; that time in a malaria-free country affected the risk of severe malaria in immigrants from Sub Saharan Africa, and after 15 years of residency, the risk for the most severe manifestations of malaria was as high as in non-immune travellers.

5.4.2 Estimating longevity of immunity to severe malaria

Although none of the previous publications specifically have assessed duration of residency in a non-endemic country, two studies of imported malaria have partly addressed the different constitution in endemic populations related to this time factor. Mascarello et al classified individuals with African origin diagnosed with *P. falciparum* as newly arrived immigrants or residents visiting friends and relatives (VFR) and found only three cases of severe malaria (WHO 2000 criteria) among 209 patients, and all in VFR that had resided in Italy for 13-19 years [187]. In contrary, Bouchaud et al compared clinical characteristics prospectively collected in European travelers (N=99) and VFR of African origin (N=252) with a median of 14 years stay in France, and observed a significant difference in frequency of severe *P. falciparum* malaria between the two groups (15.2% vs 4.4% in VFR) [143]. However, the range of years of residency in France was wide (4 - 45y), without any further stratification for time. Interestingly, they reported that the majority of VFR had visited their home-country after immigrating to Europe, indicating possible boosting of immunity. Previous visits to an

endemic country would have been interesting to include in the assessment of risk factors, but reliable data was not possible to obtain retrospectively in our patient population.

Certainly, there are additional factors with potential to modify previous immunity and risk of severe malaria. In study I, the total number of patients with severe malaria in each strata were too small in order to adjust for additional factors affecting severity, most importantly age, but we observed that immigrants from Sub-Saharan Africa with severe malaria were younger across all strata compared to the non-immune travellers, reflecting the different age profiles in the two groups. However, adjustment for age, health care delay and region of presentation in Study III reduced the effect of time in Sweden to some extent. The acquisition of life-style associated comorbidities in immigrants with long residency in Sweden could possibly also explain some of the increased risk, as discussed in a later section.

Interestingly, we observed an inverse relation of patient origin and severity among patients with non-*falciparum*, and consequently could not demonstrate any association with time in a malaria-free country. Instead we observed an increased risk among newly arrived immigrants, mostly with *P. vivax*, that constituted over 50% of severe cases in this group. Possibly different mechanisms behind development and maintenance of immunity in non-*falciparum* species could explain some of the diverging results [278], and this aspect needs further investigations, preferable in studies with larger non-*falciparum* populations and immunological approaches.

5.4.3 Serological markers assessing immunity

The results of study I+III indicate that protective immunity to severe malaria is maintained for many years but will eventually be lost without continuous exposure. As discussed previously, country of residency is only a surrogate marker for previous immunity and duration of residency in a malaria free area an estimation of unexposed time. We know from previous studies that antibodies are important for protection to clinical malaria [128], but *P. falciparum* antibody response has been shown to be short-lived [279, 280], although more recent studies have demonstrated that antibodies to certain antigens vary both in breadth, magnitude and longevity, depending on previous exposure [281]. In the study by Bouchaud et al *P. falciparum* antibodies were measured 10-12 days after treatment onset, and titers were higher in the African immigrants (with a median of 14 years of residency in France) compared to travellers born in France. However, the titers did neither correlate to duration of residency in France nor clinical outcome, and the difference in titers most likely reflects activation of immunological memory in the semi-immune population rather than circulating antibodies before infection. Ndungu et al demonstrated that long lasting memory B-cells can persist in travellers with previous *P. falciparum* infections for up to 16 years without re-exposure, but not all individuals developed detectable MBCs and we lack knowledge of their potentially protective function [141].

Ideally, we would have a marker for assessing semi-immunity in patients, and several candidates have been proposed [268], but a problem is to confirm correlation between

existence of an antibody and true clinical protection and there are still no tests in clinical practice. In addition, antibody responses involved in protecting individuals from mild and severe malaria, respectively, might differ [282].

Immuno-epidemiological studies of populations from regions with different levels of malaria transmission, and preferable long exposure-free follow up, are needed to clarify the immune responses involved in protection against severe *P. falciparum* malaria and its longevity. At the moment, duration of residency in a non-endemic country in individuals with endemic origin may be the best surrogate marker we have at the moment.

5.4.4 Recently arrived migrants

Newly arrived immigrants from endemic regions form in several aspects a unique group, differing from those that regularly are described as "VFR" –immigrants who since several years live in Sweden. Both in the Stockholm population of study I and in the national cohort of study III we observed an increased risk of severe *P. falciparum*, in study III also severe non-*falciparum,* in the group of migrants with <1 year of stay in Sweden. The association with severe *P. falciparum* was as strong as for immigrants with >14 years of stay in Sweden. For severe non- *falciparum* almost all episodes were migrants from mainly Eritrea that had arrived to Sweden within the last year, a group that has been described in detail elsewhere [38], however the majority of those with severe malaria had severe anaemia or hyperbilirubinaemia as only criterion. Other authors have also regarded newly arrived migrants as a special risk group, but mostly handled by excluding them from comparisons with non-immune and semi-immune populations [149, 188]. One case series study of imported malaria in Italy reported no severe cases among newly arrived immigrants (Mascarello), while another demonstrated an increased risk, in line with our results [194]. One might expect this group to be at lower risk of severe malaria because of recent boosting of immunity by repeated exposure, and other studies from non-endemic settings have shown that asymptomatic parasitaemia is common among recently arrived migrants [283]. However, the combination of recent exposure with an immune system possibly debilitated by undernourishment, stress and other infectious diseases after weeks of migration, might explain the increased risk of severe malaria in population migrating through areas of high transmission.

The results from study I and III reinforces the findings from study I that travellers with endemic origin are a heterogenic group with different risk of severe malaria depending on several factors other than country of birth.

5.5 SEVERITY IN NON-*FALCIPARUM* MALARIA

According to WHO criteria, over a third of all (72/227) severe malaria episodes diagnosed 1995-2015, were caused by non-*falciparum* species, and a similar proportion; 7.7%, of imported *P. vivax* cases compared to 9.4% of *P. falciparum* were severe (study III). There is

now a growing body of evidence that also non-*falciparum*, in particularly *P. vivax*, can cause severe and fatal malaria, reviewed in [91, 98, 284], with a few studies reporting comparable incidence of severe malaria in *P. vivax* compared to *P. falciparum* [284], however most reports come from endemic settings. Except for occasional case reports [101-103] , and an earlier case series study reporting only 2.4% of severe disease among imported *P. vivax* [105], studies of severe malaria in Europe have mainly included *P. falciparum*. In a review of notified cases of *P. vivax* in UK between 1987-2013, 7 deaths were reported among 12 769 cases, however with no data on severity [104].

The pathophysiology in severe *P. vivax* has been shown to differ compared to *P. falciparum* and the majority of severe *P. vivax* cases in study III presented with only one criterion of either hyperbilirubinemia or severe anaemia, which also has been reported as the most common severe signs in other patient populations with *P. vivax* [91]. Microvascular sequestration of parasitized red blood cells does evidently not play any role (Anstey 2009), but instead *P. vivax* has been demonstrated to elicit a greater inflammatory response (Andrade 2010, Anstey 2009) and cause 4-5 times greater loss of uninfected red blood cells, which partly could explain the pronounced anaemia often associated with severe *P. vivax* (WHO 2014). Nonetheless, there was a considerable proportion of *P. vivax* episodes fulfilling criteria for poor prognosis (23/72), especially among newly arrived Eritreans in 2014-2015, which of course greatly affected the overall prevalence of severe malaria in *P. vivax* cases. In this particular migrant population there was also a case presenting with coma, which although previously regarded as an unusual manifestation for *P. vivax*, lately has been observed among children in *vivax*-endemic settings [92, 93, 285]. Eight cases fulfilled the criteria of circulatory shock, which in line with other studies has been shown to be the third most common criteria reported for severe *P. vivax* malaria [91].

Recently, severe disease has also been reported in *P. ovale* and *P. malariae* infections in both endemic and non-endemic settings [98-100], also with occasional deaths in non-endemic settings [145, 181], but WHO has still not included criteria for these species in the definition of severe malaria [41]. In our nationwide cohort, 10 of the 72 severe non-*falciparum* episodes were caused by *P. ovale* and two by *P. malariae*, using the criteria for severe *P. vivax* (WHO 2015). Among these, a few also fulfilled criteria prognostic of unfavourable outcome, and two *P. ovale* needed ICU, one presenting with coma and circulatory shock, an uncommon presentation for this species, and two with pulmonary oedema which lately has been reported as a common manifestations of severe *P. ovale* [98, 99]. Of importance, none of the 12 episodes were confirmed by PCR, thus some of *P. vivax* cases could have been misdiagnosed as *P. ovale*, or vice versa, and there is also the possibility of missed mixed infections that could explain the high proportion of severe manifestations among non-*falciparum*.

Severity in mixed infections have been shown both increased and decreased prevalence depending on setting and population [88, 286, 287]. In our cohort we found the highest proportion of severe malaria among mixed infections; 21% (8/38), and even one case of death in a patient co-infected with *P. falciparum* and *P. ovale*. Again, as neither mixed nor single

infections have been systematically confirmed by PCR, we might have underestimated the total number of mixed infections, thus possibly overestimating the prevalence of severe cases among mixed infections.

Risk factors for severe non-*falciparum* malaria have previously not been systematically evaluated in a non-endemic setting. In study III we identified newly arrived immigrants from SSA, older age and longer health care delay as risk factors for severe non-*falciparum* malaria. In contrast to studies from vivax endemic settings, children were not identified as a risk group [94, 284]. In the UK review, age >70 was observed to be associated with mortality in imported *P. vivax*, however the observation was based on only seven cases with fatal outcome. Malnutrition was shown to be a risk factor for both severity and death in *P. vivax* in India [288], and possibly this state could have contributed to the large proportion of severe malaria seen among the newly arrived immigrants.

In summary, severe presentations need to be considered also in imported non-*falciparum*. And although severe anaemia, the second most common sign observed in this group, might not be considered prognostic for poor outcome, it is nonetheless associated with important consequences for health, not least in pregnant women and children [94]. Reasonably, we need to adapt the WHO severe criteria to different species and different settings.

5.6 COMORBIDITIES AND MALARIA

In paper II, we showed that comorbidity, both in terms of Charlson Index score ≥1, and multi-morbidity (having two or more chronic diseases) were associated with severe *P. falciparum* malaria, independent of age, health care delay and patient origin. Despite the numerous publications of imported malaria that have demonstrated an increased risk of severe or fatal malaria with increasing age, none of these have adjusted for comorbidities [145, 148, 183, 188, 269].

In our patient population, 30% with severe *P. falciparum* malaria had at least one comorbidity compared to 18% of the uncomplicated cases. Slightly lower proportions of comorbidities have been observed in previous reports of patients with severe imported *P. falciparum*, ranging from 14-25% [77, 84], but without comparison with non-severe cases. Possibly chronic diseases were underdiagnosed since they were not the main focus of these investigations. Since we used both medical records, additional registered ICD codes in electronic patient records, and information from medication lists on current medication, we expect to have achieved a high sensitivity in the assessment of comorbidities. A more recent multicentre review of severe malaria in Europe, reported 43% of patients having an underlying chronic disease, of which hypertension was the most frequent (9%), and possibly the prevalence of comorbidities among travellers is increasing, as suggested by other authors [189]. Unfortunately, none of these studies included comparison with uncomplicated cases.

5.6.1 Non-communicable diseases

5.6.1.1 Diabetes

When assessing individual non-communicable diseases in study II, we found that diabetes, hypertension and cardiovascular disease were all associated with severe malaria. However, after adjustments, only diabetes remained significantly associated with severity, with a three times increased odds for severe malaria according to the 2012 WHO criteria.

There are only a few studies from endemic settings that have investigated the role of non-communicable diseases in relation to malaria, however with focus on risk of infection, without assessment of severity. Two observational studies from Ghana both showed that semi-immune individuals with diabetes type II are more susceptible to asymptomatic *P. falciparum* infection [173, 174] and that the risk of infection was associated with poor glycemic control in (Danquah et al), which also was confirmed in a later cross-sectional study from Nigeria [289]. The case-control study by Danquah et al also included patients with hypertension (with and without diabetes), and those with only hypertension had slightly higher *P. falciparum* prevalence compared to disease free controls, but the increased risk for infection in diabetics was not affected by hypertension status [174].

In a hospital based descriptive study from India the prevalence of diabetes (mostly type II) among 624 prospectively collected patients with severe malaria was 17.4% [290], as compared to 9.8% in study II, however there was no comparison with non-severe cases and the background prevalence of diabetes is much higher in India compared to Sweden [291]. In addition, 10% of the patients were diagnosed with diabetes during the hospital stay, which could contribute to the high prevalence. We only included diagnoses present at admission, to prevent detection bias, and although there is the possibility of incorrectly classifying patients with undiagnosed type II diabetes as well as other diseases as healthy, this would have resulted in a non-differential misclassification.

5.6.1.2 New studies and other non-communicable diseases

Since the conduction of study II, three new studies assessing comorbidities in relation to severe imported malaria have been published. In a prospective multicentre study of imported *P. falciparum* cases conducted in France, 16.4% of severe vs 5.7% of uncomplicated malaria had at least one comorbidity and 10.5% of severe cases had immune deficiencies compared to 5.8% of non-severe, both were significantly associated with severe malaria [266]. Other comorbidities were not individually assessed and the group “immune deficiency” was not closer defined, making comparison with our study difficult. Malignancies and autoimmune diseases could both be regarded as diseases with potential to affect the immune system, but the prevalence of both disease groups was low in our study population (<1% in both groups), making inference unreliable. Of note, none of the patients with autoimmune diseases were on immunosuppressive treatment at diagnosis of malaria, and none of the recorded cancers were assessed as active.

A recently published review of 4823 severe malaria cases in USA reported similar proportion of type II diabetes (10.4%) and somewhat higher percentage of hypertension (20.4%) compared to our study population, but the prevalence among non-severe cases was insignificantly lower (6.8% for diabetes and 12.4% for hypertension), and they could not detect any association with severity [203]. An explanation to the diverging results is that only hospitalized malaria cases were included, and since comorbidities will affect likelihood of hospitalization, prevalence of diabetes among uncomplicated cases might be overestimated. In addition, the malaria cases were identified by ICD-9 discharge diagnoses, without microbiological confirmation, also severity criteria were based on ICD-9 codes, thus misclassifications could have occurred both concerning case definition and assessment of severity, possibly leading to a dilution of effects.

In another recently published study from Germany [292], comorbidities were retrospectively assessed among 536 patients with imported *P. falciparum* malaria, similar as in our study they found both that the number of comorbidities and seriousness (according to age-adjusted Charlson comorbidity index) were associated with severe malaria, using the WHO 2015 definition. In the assessment of individual diagnosis, hypertension, which was highly prevalent in the study population, cardiovascular diseases, and dyslipidaemia, were all associated with severe disease, also after adjustment. However, they found no association with diabetes, which had an overall low prevalence in the population and BMI was not assessed. Since hypertension and dyslipidaemia are seldom isolated conditions, but often associated to both hyperglycaemia and obesity [293], it would have been interesting to know what proportion of these patients also had other features of the metabolic syndrome.

5.6.2 Obesity

Only a handful studies in humans have assessed BMI in relation to malaria and these have mainly investigated overweight (BMI≥25), none the effect of obesity (BMI≥30) [175, 294, 295].

In adults admitted with uncomplicated *P. falciparum* malaria in Bangkok [175], BMI ≥ 25 was associated with development of severe malaria after treatment start. The finding could be related to under-dosage of antimalarial drugs which is not a likely explanation in study II where the majority of the obese patients with severe malaria presented with severe signs before initiation of treatment. In another study from Thailand, higher mean BMI was observed among severe *P. falciparum* compared to both non-severe cases with hyperparasitaemia >5% and mild cases with parasitaemia <5%, however the differences were small and mean BMI among severe cases was <25 [294]. Two studies, from a high and low-endemic setting respectively, reported an association of high BMI with fatal malaria, however, again mean BMI was low in these settings, and there was no data on obesity [176, 295].

We found a strong association between severe malaria and obesity (BMI ≥30), but not for overweight (BMI 25-29), also when adjusting for age, patient origin and health care delay

(adjOR 5.6). In patients with obesity and an additional metabolic risk factor (hypertension, diabetes or dyslipidaemia), the odds for severe malaria was even more pronounced (adjOR 6.5), suggesting that patients with metabolic syndrome could be at the greatest risk of severe disease. Notably, mean BMI in our study was 25.2 among uncomplicated and 29.1 among severe cases, thus not comparable to the settings described above. To our knowledge, the effect of BMI and/or obesity on severity and outcome in patients with imported malaria has not been evaluated in other studies.

An important limitation in study II is that data for weight and height was only present for patients diagnosed in Stockholm and Umeå (N=569), thus the analysis of obesity was restricted to these patients. In this group a substantial proportion of patients were also lacking data on BMI, which we handled by multiple imputation, including variables associated to missing data, and the results of the imputated analysis were similar to the complete case analysis.

Another methodological issue is the considerable overlap of diagnoses in patients with metabolic conditions. Additional adjustment for cardio-metabolic diseases (diabetes, hypertension and cardiovascular disease) did not change the association between obesity and severe malaria significantly, but adjustment for obesity did reduce the effect of diabetes somewhat, indicating that the effect of diabetes is only partly by obesity.

5.6.3 Do patient origin and time in a malaria-free country modify the effect of comorbidities?

Interestingly, both diabetes and obesity were more common among patients with endemic origin, and the highest prevalence for both conditions was seen in patients from Sub-Saharan Africa with residency of ≥15 years. When using a modified version of the stratification used in study I, we demonstrated that the strongest association with severity also was seen among patients of endemic origin with ≥15 years of stay. However, the number of patients in each strata was small and we could not verify that there was any significant interaction. Interestingly, the recently published study from Germany, also demonstrated hypertension to be more common among individuals of endemic origin, and the association with severity was independent of patient origin, however the effect of time spent in a malaria-free country was not assessed.

Possibly, life-style associated metabolic comorbidities such as diabetes and obesity, that may be acquired after many years of residency in a non-endemic country, could modify immune response, resulting in loss of previous immunity to severe malaria in endemic populations.

5.6.4 Non-communicable diseases in patients with malaria: Implications

In summary, these recent studies of risk factors for severe imported malaria, together with the results from study II, demonstrate that non-communicable diseases need to be taken into consideration in the management of malaria patients. Cardio-metabolic diseases seem to have an impact on severity in *P. falciparum* malaria, but the specific effect of diabetes has not yet

been reproduced in other settings, possibly due to differences in patient selection, background prevalence and assessment of both comorbidities and severity of malaria. Additional studies in settings of different endemicity, preferable with prospective design, where both BMI and parameters such as B-glucose and HbA1c are systematically measured, and a large patient material enabling stratification of these partly overlapping disease-groups, are needed to establish which diagnoses are of most importance.

5.6.5 General mechanisms: Obesity, diabetes and susceptibility to infections

Both diabetes and obesity are well known risk factors for susceptibility and severity of various infectious diseases [177, 296, 297], most recently recognized during the ongoing Covid-19 pandemic [298]. Proposed mechanisms for these association have been the low grade chronic inflammation characterizing both diabetes and obesity, together with altered levels of metabolic hormones and nutrients such as glucose and lipids, affecting the immune system through different pathways [299-301]. In obese individuals, poor antibody response has been observed after vaccination towards for example tetanus, seasonal influenza and hepatitis B [301]. And possibly pre-existing microangiopathy could predispose diabetics to several severe manifestations [302].

In addition to the altered immune response that has been observed in both obese and diabetic patients, availability of lipids in obese, and hyperglycemia in diabetics with poor b-glucose control, could possibly predispose to an increased risk of infection and/or higher parasitaemias with *P falciparum*, as discussed in more detail below.

5.6.6 Diabetes and hyperglycaemia – Beneficial conditions for the *Plasmodium* parasite?

In vitro studies have demonstrated that an increased availability of glucose promotes *P. falciparum* growth [303] and that the glucose flux into infected red blood cells is several fold higher than in uninfected cells [57]. In the case-control study from Ghana [174], each mmol increase in B-glucose lead to a 5% increase in risk of malaria infection, indicating that hyperglycaemia may play a biological role also in vivo.

At least some of the immune deficits observed in diabetics, have been linked to hyperglycemia and advanced glycation end products (AGEs) [304]. AGEs on erythrocytes in diabetics has also been shown to affect red blood cell deformeability and increase erythrocyte aggregation, involved in microvascular complications of diabetics [305]. This could theoretically also enhance rosetting formation which is a corner stone in pathogenesis of severe malaria.

During the course of this thesis we performed a collaborative study assessing *P. falciparum* growth and rosetting rate ex vivo in diabetics compared to non-diabetics. The results demonstrated that erythrocytes from diabetic patients are more prone to form rosettes compared to non-diabetics when cultured with *P. falciparum*. Parasite growth rates were also

significantly higher in patients with diabetes than controls, and the growth rate correlated to B-glucose levels. (Jun-Hong Ch'ng, Kirsten Moll, Katja Wyss, Ulf Hammar, Mikael Rydén, Olle Kämpe, Anna Färnert and Mats Wahlgren: Enhanced virulence of Plasmodium falciparum in diabetic patients, manuscript under submission).

Interestingly, in a post-hoc analysis of patients in Stockholm (study II) we found that 83% diabetics with severe malaria had poor B-glucose control (defined as elevated HbA1c or fasting blood glucose the year of malaria diagnosis) compared to 33% diabetics with non-severe malaria. However, the patients with poor glucose control did not have higher parasitaemias, and although the criteria of hyperparasitaemia >5% was more common among diabetics than non-diabetics, the difference was not significant.

Biguanides used by many type II diabetics has been shown to possess antimalarial effect [306] which could have underestimated the association observed for diabetes, but we found that these medications were similarly used in diabetics with severe and non-severe malaria (study II).

5.6.7 Obesity, lipids and animal models

Metabolic changes in the obese could have implications for the *Plasmodium* parasite as well. The availability of lipoproteins affects the building of parasite cell membranes and endothelial adherence of infected erythrocytes [307-309]. In the unpublished study by Ch'ngH et al parasite growth correlated to serum-triglyceride levels *in vitro*, and in vivo, dyslipidaemia has recently been associated with severe malaria (Hoffmeister 2019). The role of hyperlipidaemia in relation to obesity's association with severity would have been interesting to investigate, but we lacked information on lipid status, and dyslipidaemia was an uncommon comorbidity in our study population.

A significant greater proportion of obese patients had hyperparasitaemia compared to non-obese, and extreme parasitaemias of >10% accounted for the majority of this difference, a feature also described in obese mice and rats (Lombard 1998, Robert 2008[310, 311]). However, as for diabetes, parasitaemia could not solely explain the more severe presentation in obese, as most patients also had other severe signs than hyperparasitaemia.

5.6.8 Is diabetes and obesity associated to specific severe manifestations?

From an etiological perspective, potential associations with individual clinical presentations of severe malaria would be interesting to investigate for both diabetes and obesity, however such subgroup analysis would have been underpowered in our study. The proportions of individual criteria among diabetics and obese compared to non-diabetics and non-obese has been presented in supplementary material of Study II, and the comparison indicates that respiratory distress, pulmonary oedema, and renal impairment were common manifestations among diabetics. Although these manifestations also were common among diabetics with severe malaria in the study by Khuu et al, they could not demonstrate any increased risk for specific manifestations [203]. In contrast, a hospital based study in India found a higher

proportion of multi-organ involvement among diabetics with severe malaria compared to severe cases without malaria [290]. Common severe presentations among the obese were slightly different from patients with diabetes, and included circulatory collapse, pulmonary oedema and acidosis.

Evidently, there is a need to further investigate the pathology of *Plasmodium falciparum* infection in individuals with obesity and diabetes, taking into account both plasma levels of glucose and lipids, any ongoing treatment, and including assessment of individual severe criteria in relation to different comorbidities.

5.6.9 Communicable diseases – HIV and malaria

HIV has repeatedly been described as a risk factor for a more severe disease course in *P. falciparum* malaria, especially in pregnant women and HIV positive patients with low CD4 count [18, 167], but also in non-immune travellers with imported malaria [191, 203, 312]. HIV-infection has also been proposed as a risk factor for severe *P. vivax* [94], although there are only occasional case reports supporting this [313].

In both study II and III we observed an increased risk for severe *P. falciparum* malaria in patients with HIV, despite that most of these patients had a treated and well-controlled HIV infection. The total number of patients with HIV was rather small in both studies and HIV was not included in multivariable analysis to avoid drift in ORs. Also, in study II, the association with HIV became non-significant after excluding hyperparasitaemia as single criterion in the severe malaria definition, indicating that some of the association with severity can be attributed to hyperparasitaemia, and higher parasitaemia were also observed among patients with concurrent HIV and *P. falciparum* infection in malaria-endemic settings [314, 315].

Among patients with non-*falciparum* malaria (study III), there was only one patient with known HIV and severe malaria (*P. ovale*), thus we could not show any association for severe non-*falciparum* malaria.

An important limitation in the assessment of HIV is that there was no systematical screening for HIV in malaria patients, but possibly, testing in conjunction with the malaria episode could have occurred more often in severe cases, which would result in a detection bias. However, in study II we reviewed the HIV cases and found that 18 of 21 were known before malaria diagnosis, and none of the three new detected HIV infections were in severe malaria patients.

5.7 LONG-TERM EFFECTS –LYMPHOID NEOPLASMS AND OVERALL RISK OF CANCER

Study IV is the first register-based study that investigates long term risk of lymphoid neoplasms and other cancers in patients diagnosed with malaria. We showed that a single episode of malaria in travellers was not associated with an increased risk of lymphoid neoplasms. However, for malaria patients born in Sub-Saharan Africa, there was a two-fold

increased risk of lymphoid neoplasms. There was no increased risk of other cancer in neither travellers nor immigrants diagnosed with malaria.

5.7.1 Malaria and lymphoma

There is a well-known epidemiological link between *P. falciparum* malaria and Burkitt lymphoma in children living in endemic Sub-Saharan Africa [213-215]. Endemic Burkitt lymphoma (eBL) is also seen in children living in Papua New Guinea [316], where transmission of *P. falciparum* is similar to that in holoendemic areas of Africa. A few studies indicate that malaria also is involved in paediatric Burkitt lymphoma in regions of Brazil with high incidence of *P. falciparum* [317]. The association is largely based on ecological studies that have matched geographical differences in malaria transmission with incidence of lymphoma, thus a causal relationship has not been established. Further evidence has been brought by case-control studies demonstrating high titers of *P. falciparum* antibodies in cases of Burkitt lymphoma (BL) [318, 319] and genetic studies showing an inverse relationship between sickle cell trait and eBL [320-322]. In a recent nested case-control study from UK also the non-endemic (sporadic) form of BL was associated with previous malaria exposure, however, in this study malaria exposure was defined as either a treatment for malaria, a reported history of malaria, or a receipt of malarial prophylaxis, thus not a confirmed malaria diagnosis [323].

A few reports from endemic countries have suggested a link also with non-Burkitt lymphoma [216, 217, 324]; and in three earlier case-control studies from non-endemic regions, previous malaria was associated to non-Burkitt non-Hodgkin lymphomas [325, 326] and low-grade lymphatic malignancies [327]. However, again assessment of exposure was based on self-reported history of malaria, thus prone to recall bias.

In study IV, B-cell lymphomas dominated among malaria patients with lymphoid neoplasms and the association became stronger when using a stricter definition of lymphoma, excluding myeloma, CLL and ALL. Because of the small total number of lymphoid neoplasms, it was however not meaningful to assess associations with individual lymphoma subgroup. Interestingly, there was only one Burkitt lymphoma in the whole cohort of malaria patients and matched population comparators, and it was diagnosed in a 52-year old with confirmed malaria, endemic origin and no known HIV.

5.7.2 Malaria and cancer

Evidence is scarce regarding association between malaria and other cancers. In Uganda, cervical cancer was found to be more common in women living in areas with high *P. falciparum* endemicity [220]. While one study from Vietnam found no significant association between malaria and primary hepatocellular carcinoma [328], another study from Southeast Asia reported an association between nasopharyngeal carcinoma and previous malaria [329]. Malaria has also been proposed to play a possible role as a co-factor in HHV-8 (Human Herpesvirus-8) tumorigenesis in Kaposis sarcoma in non-HIV-patients [330]. Again, these

studies lack longitudinal design, and most were either based on geographical associations or self-reported history of malaria in cases of cancer.

We did not detect any increased risk for all-site non-haematological cancer in malaria patients, independent of birth region. However, there was a tendency of lower risk of other cancers in individuals born in SSA. We did not assess the association with individual cancers, due to small numbers in each subgroup and multiple testing would have resulted in unreliable estimates. We did however present proportions of different cancers (all site) in the cohort and, interestingly, the proportion of individuals with female genital tract cancers (mainly cervical and placental tumours) was higher among individuals from SSA diagnosed with malaria compared to matched comparators without malaria. There were some expected differences related to birth region and well known carcinogenic risk factors, such as lower proportion of melanoma but higher proportion of liver cancer and endocrinal tumours (mainly thyroid gland) among individuals born in SSA, but none of these were associated with previous malaria exposure.

5.7.3 Limits and advantages of a register based study

Population based studies have to our knowledge not been used before to investigate long-term consequences of malaria. A major strength of study IV is its nationwide coverage, long-term follow up and high quality registers, providing a near complete and unbiased detection of cancers [248]. In addition, malaria patients were identified by several modes including Sweden's surveillance system of communicable diseases with compulsory notification, complemented with the National patient registers and medical records to detect non-notified cases. As an additional analysis we included all Swedish residents with birth country in Sub-Saharan in order to provide a robust comparator population for the patients with endemic origin. As discussed previously, estimating previous exposure based on country of birth will not be fully accurate since malaria transmission can vary greatly within a country as well as change over time. In an attempt to improve our estimates of level of exposure, we included total number of years lived in an endemic region in addition to birth country in our analysis. This revealed a tendency of increased risk for lymphoma in individuals with over 30 years of stay, however we could not demonstrate that time in endemic country modified the risk associated with a malaria diagnosis. We interpret the findings such that, being diagnosed with malaria in Sweden might be a stronger indicator of previous high malaria exposure in individuals originating in Sub-Saharan African.

The setting in a non-endemic country prevents reinfection within the country, but there are certainly individuals diagnosed with malaria outside Sweden that have not been captured, especially in the migrant population with endemic origin. In addition, there were approximately 1350 immigrants with reported malaria that we could not follow due to the lack of personal identification numbers. These mostly constituted of temporary visitors in Sweden but could also include recently arrived migrants diagnosed with malaria that later could have become residents in Sweden, thus possibly incorrectly misclassified as unexposed. The misclassification of exposure together with the small number of lymphoid

neoplasms in patients with endemic origin are probably the most important limitations in study IV.

A reassuring sign however was that when only microbiologically confirmed or notified cases were included the association with lymphoid neoplasms became stronger. Also the misclassification of exposure is non-differential and would only dilute the association observed.

5.7.4 Interpretation of findings and possible mechanisms

While the carcinogenic role of EBV in lymphoma development is well described, malaria has been proposed to have a more co-pathogenic role involving general immunosuppressive effects, promoting EBV-reactivation leading to increased EBV viral loads, as demonstrated in children with acute [331] as well as recurrent *P. falciparum* infections in holoendemic malaria regions [332]. In animal models different *Plasmodium* species have shown to promote growth of both lymphoid [333, 334], and non-lymphoid tumours [335], often with the involvement of oncogenic viruses. However, recently, there are also indication that *P. falciparum* parasites could have more direct mutagenic effects, by inducing dysregulated expression of the DNA-mutating enzyme AID (activation-induced cytidine deaminase) resulting in B-cell proliferation and activation and increasing likelihood of c-myc translocation that characterizes Burkitt lymphoma [336, 337].

Considering that reactivation of oncogenic virus via general immunosuppressive effects of concurrent malaria infection is a proposed mechanism in the development of endemic Burkitts lymphoma, it would have been interesting to control for previous exposure by EBV and possibly also other oncogenic viruses. However, although we could have used discharge diagnoses for mononucleosis, this would be far from a true marker of previous EBV infection considering the large proportion of asymptomatic infections, as well as lack of data for newly arrived immigrants. Attaining serologies for all patients would not have been manageable and EBV sero-prevalence is anyway estimated to be >90% in most adult populations.

In line with some recent studies that have assessed antibodies to several different *P. falciparum* antigens [338], as well as demonstrated a greater genetic diversity of *P. falciparum* infections in eBL cases [339], it seems likely that recurrent infections, probably with several different strains, contribute to lymphoma development. Supposing a similar mechanism for other B-cell lymphoma, this would explain why the association with lymphoid neoplasms in study IV only could be demonstrated in patients who were born and had spent over 20 years in malaria endemic regions where exposure likely has occurred repeatedly during childhood and adolescence. Consequently, we could not demonstrate any additional risk for patients with severe malaria, that in our non-endemic setting mainly constitutes of non-immune travellers with first time malaria.

The association with eBV in children has only been observed for *P. falciparum* infections, possibly due to *falciparum* specific antigens (PfEMP1) binding to B-cell receptors involved in polyclonal B cell activation and expansion of B cells with latent EBV [340]. Consistently,

the majority of malaria patients who developed lymphoma in study IV had been diagnosed with *P. falciparum*, but there were also occasional non-*falciparum* infections among the lymphoma cases. Since we however do not consider single infection diagnosed in Sweden as the primary cause of lymphoma development, but rather repeated previous infections, this finding may be less contradictious. And among the malaria patients of endemic origin that developed lymphoma, only one had a non-*falciparum* infection, this individual however also had HIV and developed Hodgkins, thus less likely related to malaria in this case.

Study IV does not provide any mechanistic explanation for the association of malaria and lymphoid neoplasms. Although results from recent studies indicate that Plasmodium parasites could have direct mutagenic effects through AID activation in the development of Burkitt lymphoma, further studies are needed both to replicate our observations in larger populations and to provide molecular evidence for the association observed with other lymphoma.

5.7.5 Important confounders and other methodological considerations

An important challenge in study IV was the large proportion of immigrants from endemic regions, with lacking information on previous diseases as well as incomplete follow up due to emigration. Approximately a fourth of malaria patients of endemic origin had a residency in Sweden of <1 year before diagnosis, and it was therefore not meaningful to adjust for previous comorbidities, most importantly inflammatory and autoimmune diseases that has been associated to lymphoma [341]. However, there is no evidence suggesting that inflammatory diseases would be more common among individuals travelling to or immigrating from malaria endemic areas. Of more importance for this study; HIV [342] and chronic hepatitis C [343], to some extent also chronic hepatitis B [344] have been associated to lymphoid neoplasms. Since patients presenting with fever after travels to the tropics often are investigated for other infectious diseases than malaria, this group will be more well-screened for HIV and hepatitis which explains the higher prevalence of these co-infections among malaria patients in study IV. To adequately adjust for this, we chose to do an additional analyze excluding all cases with previous HIV and hepatitis, and censoring individuals with HIV or hepatitis during follow up. Despite the exclusion of three lymphomas in malaria patients, and one of these with endemic origin, this did not affect the risk for lymphoid neoplasms observed in malaria patients with endemic origin. Since patients with lymphoid neoplasms in Sweden are systematically screened for HIV and hepatitis before treatment [345], and we included HIV and hepatitis diagnosed up to 6 months' post lymphoma diagnosis in the censoring, the detection rate of these infections among individuals with lymphoid neoplasms is estimated to be high.

Patients with malaria may differ from the general population in many aspects, such as socioeconomic status and perceived barriers to healthcare access. Presumable, immigrants diagnosed with malaria in Sweden would be more likely to seek medical care in Sweden for other diseases and symptoms, thus increasing the probability of detecting lymphoma. However, since the risk for other cancers rather was lower in this group, this is an unlikely explanation for the association with lymphoma. The same applies for other factors that may

have confounded the results, such as differences in other traditional risk factors for cancers such as alcohol and smoking.

A “healthy migrant effect” as well as a “salmon effect” (described in the methods section of this thesis) could contribute to the overall lower cancer incidence observed in the Sub-Saharan African cohort and would thus mean that the incidence of cancers is underestimated in this population. These methodological explanations do however not fit with the observed higher incidence of lymphoid neoplasms in the subset of population with previous malaria diagnose.

The possibility of a joint unknown risk factor, such as an another exposure, or a genetic trait related to both malaria infections and lymphoid neoplasm development, or reverse causation; involving higher probability of symptomatic malaria in semi-immune individuals with an undiscovered lymphoma, cannot be entirely excluded. However, we have tried to minimize such bias by not including lymphomas diagnosed within the first 6 months of a malaria episode, in addition the majority of the lymphoid neoplasms were diagnosed over 5 years after malaria. Still, we can never completely rule out residual or unmeasured confounders that we have not been able to control for.

5.7.6 Global cancer burden

Cancer incidence pattern in Sub-Saharan Africa shows several striking differences compared to the rest of the world; with higher incidences of cancers related to infectious diseases (liver cancer, cervical cancer, Kaposis sarcoma, NHL) and as much as one third of the cancers in this region are estimated to be related to infectious disease agents [207, 346]. As demonstrated by a study from USA, this pattern seems to remain to certain extent also in immigrant populations. [347].

The identification and elimination of cancer related infections are thus of great importance for public health in Sub-Saharan Africa, and the results of this study further stresses the importance of reducing malaria transmission in high-endemic areas.

6 IMPLICATIONS

The studies of this thesis identified several risk groups for severe malaria that might need special attention. Some of these, such as pregnant women and patients of old age have been recognized in previous studies. However, immigrants of endemic origin are often regarded as being at lower risk of severe manifestations. Here we clearly showed that both newly arrived migrants and immigrants with long residency should be considered as potential risk groups for severe malaria, and not only caused by *P. falciparum*. As manifested by the extraordinary surge in 2014-2015 of *P. vivax* among Eritrean immigrants, and our findings of a high proportion of severe manifestations in this group, a screening program for certain migrant population might be warranted, and in an ongoing study in our research group, the prevalence of *Plasmodium* infections among recently arrived migrants from Sub Saharan Africa is being investigated.

Moreover, we demonstrated that time of residency in Sweden affected the risk of severe malaria in immigrants from Sub Saharan Africa, adding to the understanding of the longevity of immunity to severe malaria. Considering the change in malaria transmission seen in many parts of the world, the results could have implications for populations at risk, as protection to severe malaria may change with declining exposure. In addition, clinician need to be aware of the possibility of severe presentations in migrants regarded as semi-immune.

One of the challenges with malaria in a non-endemic setting is to suspect the diagnosis. Health care delay was observed in a third of the severe cases and in two out of four deaths, and was more common in children, women and travellers of non-endemic origin. In addition, health care delay was associated with severity in both *P. falciparum* and non-*falciparum* malaria. Thus, awareness of imported malaria as differential diagnosis can evidently be improved.

Travellers of older age and with multiple comorbidities are also visiting countries where there is substantial risk of being exposed to malaria. At the same time, regions with high malaria burden are experiencing a change in disease panorama, with an increasing prevalence of non-communicable diseases (NCDs). Despite this, NCDs potential impact in populations at risk of malaria have been little investigated. In this thesis we showed that diabetes, obesity and a combination of metabolic risk factors increased the risk of severe *P. falciparum* infections, but additional studies are needed to assess if the results are generalizable to other parts of the world, in particular Sub Saharan Africa.

The definition of severe malaria is complex, as has been discussed throughout this thesis. Better adapted criteria are needed for the non-endemic setting, possibly also for endemic countries. Further in depth studies using our clinical data set could identify even better which signs and manifestations are relevant for identifying those patients that are in most need of a higher level of care. Our results on risk of deteriorating with oral treatment at higher parasitaemias has already been incorporated in local guidelines and i.v. treatment is now suggested for parasitaemia >2% in the Swedish national recommendations. Possibly, scores used in general for predicting outcome in the ICU setting, could aid the management of

patients with severe malaria. In addition to the clinical presentations and signs of the severe criteria, underlying host factors, identified in the studies of this thesis, should be considered to be included in the assessment of risk.

Finally, we have found an increased risk of lymphoid neoplasms, in individuals from endemic areas diagnosed with imported malaria, and probably heavily exposed during childhood. These findings confirm previous smaller studies, with lack of longitudinal design, proposing malaria as a risk factor for various lymphoma and indicate that clinicians might need to recognize lymphomas in malaria patients from endemic regions, many years after exposure.

Identifying long-term effects of malaria is crucial for a more complete assessment of the global burden of malaria and with 200 million people affected by the infection worldwide each year, post-malaria sequelae will have substantial effects on both morbidity and mortality, further emphasizes the need to reduce also asymptomatic malaria in high-transmission regions of the world.

The findings from these studies can hopefully aid in improving the management of patients with imported malaria, and may also have implications for management and preventive strategies in countries with ongoing transmission.

7 CONCLUSIONS

Malaria with severe presentations and intensive care were mainly caused by *P. falciparum,* but a third of the severe episodes were caused by other species, some of these also with the most severe signs.

Severe malaria was associated with different risk factors depending on species.

Age ≥60 years was the strongest predictor for severe malaria in all species, however for *P. falciparum* the association was observed starting at 40 years of age, with an increased risk for each decade of attained age.

The youngest children aged <5 years, pregnant women and patients with HIV, were also at increased risk of severe *P. falciparum* malaria.

Recently arrived migrants from Sub Saharan Africa was demonstrated to be a new identified risk group for both severe *P. falciparum* and non-*falciparum* malaria.

Health care delay was associated with severity for both *falciparum* and non-*falciparum,* for *P. falciparum* the association was observed already from one day of delay.

The overall proportion of ICU admission in episodes of severe malaria based on WHO criteria was low, while specific severe signs prognostic for poor clinical outcome better predicted admission to ICU.

Episodes of *P. falciparum* that progressed to severe malaria during oral treatment had a higher parasitaemia compared to episodes without deterioration, and patients presenting with >2% parasitaemia should be considered for i.v. treatment with artesunate.

Immigrants from malaria endemic areas who had lived in Sweden for ≥15 years were at similar risk of severe *P. falciparum* malaria as non-immune travellers, supporting the hypothesis that clinical immunity to severe malaria wanes with increasing time without re-exposure.

Travellers with more than one comorbidity are at increased risk of severe malaria when infected with *P. falciparum.*

Obesity, diabetes and combinations of metabolic risk factors were highly associated with severe *P. falciparum* malaria in both non-immune travellers and immigrants from malaria endemic countries.

Individuals born in malaria endemic areas and diagnosed with malaria in Sweden have an increased risk of lymphoid neoplasms, especially B-cell lymphoma. There was no association with other cancer, nor did single malaria episodes confer an increased risk in travellers.

8 FUTURE PERSPECTIVES

As demonstrated within this thesis, studying malaria in travellers and migrants can contribute with important knowledge. Sweden has many advantages for this purpose, such as national surveillance, with high precision in detection of cases, absence of continuous re-exposure, reliable health recording system and unique population registers enabling long-term follow-up.

Moreover, considering the increasing prevalence of non-communicable diseases, especially diabetes, across the African and Asian continent, and our findings of severe malaria in patients with metabolic comorbidities, there is a need to study how these diseases can affect malaria also in an endemic setting. Clinical observations from Tanzania and Cameroon indicate that diabetes plays a role in severity of malaria infections in these countries as well, but there is yet no published data. In collaboration with researchers from Cameroon, we are planning to perform a prospective study with the aim to investigate if diabetes is associated with severe malaria also in an endemic setting. In addition, BMI would be included in the assessment, to enable estimation of the attributable risk from diabetes, obesity and other metabolic risk factors, as well as HIV. As a second step, preventive measures might be indicated, such as information to risk groups and screening, both for increased glucose tolerance among malaria patients, and for *Plasmodium* parasites in diabetics. Possibly, there is a need for implementation of intermittent preventive treatment, similar as used in pregnant women, in malaria high-transmission areas.

The mechanisms behind the increased risk of severe malaria among both diabetics and obese need further exploration. Within the small ex vivo follow up study discussed in this thesis, we have only touched upon the possible effects of glucose and lipids together with atypical properties of red blood cells promoting aggregation in diabetic patients. Certainly more studies are needed to elucidate which mechanisms are most important for the clinical outcome in patients with different comorbidities, an exciting field since it also can have implications in the treatment of severe malaria.

Long-term consequences due to malaria infections have received insufficient attention. Although neurological sequelae in children following cerebral malaria are well described, and recent studies also have shown cognitive effects after uncomplicated disease, neuro-cognitive impairment in adults have been little studied, and for travellers there are only occasional case reports, thus there is a need for more research also in this area.

In addition to cerebral malaria, severe malaria causes several other acute manifestations and considering the sequestration properties of *P. falciparum* in many different sites, there are certainly acute organ effects also in less severe malaria. Recently, chronic effects on kidney function have been shown after malaria with renal impairment in African children, also with serum creatinine at levels lower than the WHO criteria (Conroy 2019). It would thus be of great interest to investigate if the same applies for adults, as well as to further explore if different species of malaria, both with or without severe manifestations, could be associated

with other chronic diseases such as liver failure, cardiovascular diseases, neuro-psychiatric disorders or cognitive impairment manifested as long term effect on working ability. With the national malaria cohort assembled in study IV, and the unique linkage to various health and population registers, as well as to extensive clinical data collected for study I-III, we have an exceptional opportunity to investigate these and other possible long-term effects, as well as to assess if specific severe manifestations are linked to corresponding long-term organ failure. This could have importance for optimizing the future management and preventing chronic manifestations in malaria patients.

Finally, cancer is an emerging public health problem in Sub Saharan Africa, with one third of the cancers in this region estimated to be related to infectious disease agents. Thus, there ought to be great opportunities to reduce both morbidity and mortality due to cancer in Sub-Saharan Africa. We already know that *P falciparum* is associated with the most common childhood cancer in Sub Saharan Africa, and although more investigations certainly are needed, the results of study IV indicate that repeated infections in those that grow up in an endemic area also could predispose to lymphoma development later in adulthood, further emphasizing the need to strive for global elimination of malaria.

9 POPULÄRVETENSKAPLIG SAMMANFATTNING

Malaria är en potentiellt dödlig infektion där snabb och korrekt handläggning i många fall är avgörande. De flesta som drabbas av malaria bor i Afrika Söder om Sahara, men ungefär hälften av världens befolkning lever i områden där det finns risk att bli smittad. Enligt Världshälso-organisationens (WHOs) senaste årsrapport insjuknade 228 miljoner människor globalt och 405 000 avled pga. malaria. Sjukdomen orsakas av en myggburen parasit av släktet *Plasmodium* varav det finns 5 arter som främst infekterar människan. *Plasmodium falciparum* är den art som står för flest fall av allvarlig malaria och även majoriteten av dödsfall. Symptomen vid en malariainfektion är ospecifika och kan likna en influensa men i vissa fall utvecklas en allvarlig sjukdomsbild med sviktande organfunktioner.

I Sverige ser vi pga. ökat resande och migration ca 100 fall av malaria per år, med en tillfällig ökning till 350 fall år 2014. Trots Folkhälsomyndighetens nationella registrering och statistik saknas data över klinisk bild, handläggning och utfall av malaria i Sverige. Det är också ofullständigt kartlagt vilka som löper en ökad risk att drabbas av allvarlig malaria, samt om en genomgången malariainfektion har några långtidseffekter på hälsan.

Individer som växer upp i endemiska områden och exponeras för malaria under barndomen utvecklar en viss immunitet, först mot allvarlig sjukdom, och vid upprepad exponering även mot symptomgivande malaria. Det är dock okänt hur lång tid skyddet mot allvarlig malaria bibehålls hos individer som inte längre exponeras för infektionen. I och med den minskade incidensen i flera endemiska områden finns farhågor att immuniteten försämras och att man kommer få se fler fall av allvarlig malaria med dödlig utgång. Vilka kommer då att drabbas? Välfärdssjukdomar såsom diabetes, hjärtkärlsjukdom och fetma ökar i hela världen, även i malaria-endemiska länder. Samtidigt ser vi att både äldre och personer med kroniska sjukdomar i utökad utsträckning reser till tropikerna. Hur dessa sjukdomar påverkar en malariainfektion har inte klarlagts.

Långtidskonsekvenser efter malariainfektioner är också ofullständigt studerade. Det finns ett väletablerat samband mellan malaria och Burkitt lymfom hos barn i endemiska Afrika. Men det har inte undersökts om malaria ökar risken för andra typer av lymfom eller övrig cancer hos vuxna. Utvärdering av långtidseffekter är dock svåra att genomföra i malariaendemiska länder där uppföljningen är begränsad.

Syftet med detta doktorandprojekt var att identifiera riskfaktorer för allvarlig malaria samt undersöka långtidskonsekvenser efter en malariainfektion. De frågor vi önskade besvara var:

Påverkar födelseland och antal år bosatt i Sverige risken för allvarlig malaria bland immigranter från Afrika? (studie I)

I det första projektet jämfördes resenärer födda i Sverige med resenärer och migranter födda i endemiska områden i Afrika avseende risk för allvarlig malaria. Vi inkluderade 501 vuxna med *P. falciparum* infektion diagnosticerade i Stockholm under 1995-2013. Ingen skillnad avseende risk för allvarlig malaria sågs överlag vid jämförelse mellan de två grupperna, men

när patienter från endemiska områden delades upp avseende tid de bott i Sverige, observerades att bland de som hade varit bosatta i Sverige ≥15 år var allvarlig malaria lika vanlig som bland icke-immuna resenärer. Sammanfattningsvis tyder resultaten på att immunitet mot allvarlig malaria hos tidigare malaria-exponerade individer kan bibehållas under många år, men att skyddet försvinner i frånvaro av förnyad exponering. Att förstå varaktigheten av immunitet mot allvarlig malaria är en viktig kunskap både för riskbedömning av malariapatienter i Sverige och för populationer i områden där transmission av malaria förändras.

Kan kroniska sjukdomar och övervikt öka risken för allvarlig malaria? (studie II)

I den andra studien utökades studiepopulationen till att inkludera 937 vuxna diagnosticerade med *P. falciparum* malaria i större delar av Sverige under 1995-2015. Vi fann att kroniska sjukdomar var vanligare bland patienter med allvarlig malaria (30 %) jämfört med okomplicerade fall (18 %). BMI var också högre bland de allvarliga fallen. Diabetes var förenat med en tre gånger ökad risk för allvarlig malaria och vid kraftig övervikt (BMI≥30) sågs en nästan sex gånger ökad risk. Risken för allvarlig malaria var ännu mer förhöjd vid en kombination av metabola riskfaktorer. Sambanden observerades hos både svenskfödda resenärer och immigranter från Afrika Söder om Sahara. Samtida sjukdomar, och särskilt diabetes och kraftig övervikt, bör således tas i beaktning vid bedömning och handläggning av en patient med malaria.

Vilka patienter får allvarlig malaria i Sverige?? (studie III)

I denna studie utvärderade vi riskfaktorer för allvarlig sjukdom hos både barn och vuxna med olika arter av malaria, diagnosticerade i hela Sverige under 1995-2015. Sammanlagt inkluderades 2653 episoder av malaria i analysen, varav nästan 10 % (N=227) hade allvarlig malaria. De flesta fallen av allvarlig malaria orsakades av *P. falciparum* men en oväntat stor andel (32 %) sågs även hos de andra arterna. Hos ca en femtedel av fallen blev diagnos och behandling fördröjd, och detta var förenat med en högre risk för allvarlig malaria, oavsett art. För *P. falciparum* hade även små barn (<5 år), gravida, äldre, resenärer födda i Sverige och patienter med HIV, en ökad risk för allvarlig sjukdom. Nyanlända migranter från endemiska områden visade sig vara en extra utsatt riskgrupp för allvarlig malaria orsakat av alla arter. Trots tillgång till avancerad intensivvård och rätt behandling, dog fyra patienter till följd av malaria. Denna studie visar tydligt att medvetenheten om malaria inom sjukvården, liksom den initiala handläggningen, kan förbättras, möjligen med särskilda insatser för de riskgrupper vi identifierat.

Kan malaria öka risken för lymfom eller annan cancer? (studie IV)

Som sista del i doktorandprojektet har vi gjort en registerbaserad kohortstudie där samtliga malaria fall diagnosticerade i Sverige 1987-2015 har kopplats ihop till flertal olika nationella hälso- och populationsregister. 4206 patienter med malaria följdes under många år och jämföras med ett stort antal kontroller från Sveriges befolkning matchade för ålder, kön och födelseregion, samtliga avidentifierade. För individer födda i Afrika Söder om Sahara som

diagnosticerats med malaria i Sverige sågs en ca två gånger ökad risk för lymfom. Bland resenärer som endast haft malaria en gång observerades däremot ingen ökad risk och vi såg inte heller någon ökad risk för andra former av cancer. Eftersom patienterna från Afrika sannolikt haft malaria flera gånger tidigare i livet, tolkar vi resultaten som att upprepade malariainfektioner under uppväxten kan öka risken för lymfom många år efter exponering. Långtidseffekter som dessa kan ha betydande effekter för global hälsa med tanke på det stora antalet människor som infekteras årligen. Medvetenheten kring följdsjukdomar behöver öka och insatser för att förebygga även mild malaria prioriteras.

Sammanfattningsvis har vi med dessa studier identifierat riskfaktorer för allvarlig malaria som inte uppmärksammats tidigare, bl.a. diabetes och kraftig övervikt. Vi har visat att immunitet mot allvarlig malaria avtar efter många år i ett malaria-fritt land, och migranter som bott lång tid i Sverige, men även nyanlända, hade en ökad risk för allvarlig malaria. Slutligen har vi påvisat en ökad risk för lymfom hos patienter med malaria som är uppvuxna i endemiska områden. Vi hoppas att resultaten kommer bidra till optimerad handläggning och uppföljning av patienter med malaria, samt förebyggandet av allvarlig och dödlig malaria, inte bara i Sverige utan även globalt.

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